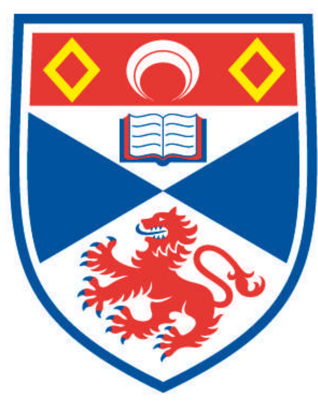
THE EFFECTS OF TEMPERATURE AND SIZE ON SWIMMING IN FISH

Stephen D. Archer

A Thesis Submitted for the Degree of PhD at the University of St Andrews



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THE EFFECTS OF TEMPERATURE AND SIZE ON SWIMMING IN FISH.

BY

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UNIVERSITY OF ST. ANDREWS.
1989.

A thesis submitted for the degree of

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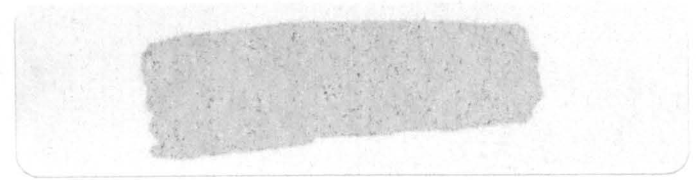
DECLARATION.

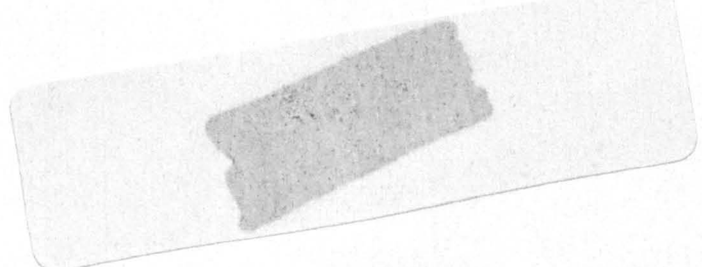
I hereby declare that the research reported in this thesis was carried out by me and that the thesis is my own composition. No part of this work has been previously submitted for a higher degree.

The research was conducted in the Department of Biology and Preclinical Medicine, United College of St. Salvator and St. Leonard, University of St. Andrews, under the direction of Prof. I.A. Johnston.



CERTIFICATE.

I hereby certify that Stephen Archer has spent eleven terms engaged in research work under my direction, and that he has fulfilled the conditions of General Ordinance No. 2 (Resolution of the University Court No. 1, 1967) and that he is qualified to submit the accompanying thesis for the Degree of Doctor of Philosophy.



"One of my good friend said, in a reggae rhythm,
don't jump in the water if you can't swim.
The power of philosophy floats thru my head
you're light like a feather,
light like a feather, heavy as lead."

Marley and the Wailers: "Misty Morning"

This thesis is dedicated to my parents,

Janet and Bob Archer.

ACKNOWLEDGEMENTS.

I am indebted to Professor Ian Johnston for his constant help, guidance and enthusiasm throughout the production of this thesis.

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SUMMARIES.

Chapter 1. General Introduction.

This study examines swimming in fish as a function of the performance of the locomotor musculature. Aspects of evolutionary adaptation and scaling relevant to swimming performance in fish are introduced. The concepts of resistance and capacity adaptations are illustrated in relation to the evolution of the suborder Notothenioidei to the antarctic environment. Alterations in hydrodynamics, swimming performance and efficiency with growth/scaling are discussed.

Chapter 2. Muscle fibre types and metabolism in post-larval and adult stages of notothenioid fish.

A histochemical study was carried out on muscle fibre types in the myotomes of post-larval and adult stages of seven species of notothenioid fish. There was little interspecific variation in the distribution of muscle fibre types in post-larvae. Slow fibres (diameter range 15-60μm) which stained darkly for succinic dehydrogenase activity (SDHase) formed a superficial layer 1-2 fibres thick around the lateral surface of the trunk. In all species a narrow band of very small diameter fibres (diameter range 5-62μm), with only weak staining activity, occurred between the skin and slow fibre layer. These have the characteristic of tonic fibres found in other teleosts. The remainder of the myotome was composed of fast muscle fibres (diameter range

9–75μm), which stain weakly for SDHase, α-glycerophosphate dehydrogenase, glycogen and lipid.

Slow muscle fibres were only a minor component of the trunk muscles of adult stages of the pelagic species *Champsocephalus gunnari* and *Pseudochaenichthys georgianus*, consistent with a reliance on pectoral swimming during sustained activity. Of the other species examined only *Psilodraco breviceps* and *Notothenia gibberifrons* had more than a few percent of slow muscle in the trunk (20–30% in posterior myotomes), suggesting a greater involvement of subcarangiform swimming at cruising speeds. In contrast to previous studies, no significant differences were found in mean diameter between fibres from red-blooded and haemoglobinless species.

The activities of key enzymes of energy metabolism were determined in the slow (pectoral) and fast (myotomal) muscles of *N. gibberifrons*. In contrast to other demersal antarctic fish examined, much higher glycolytic activities were found in fast muscle fibres, probably reflecting a greater endurance during burst swimming.

Chapter 3. Kinematics of labriform and subcarangiform swimming in the antarctic fish, *Notothenia neglecta*.

The kinematics of labriform and subcarangiform swimming have been investigated for juvenile (7–8 cm) and adult (27–30 cm) stages of the antarctic teleost, *Notothenia neglecta* Nybelin at 1–2° C. Upper threshold

speeds using the pectoral fins alone (labriform swimming) were 0.8 Ls^{-1} in adult fish and 1.4 Ls^{-1} in juveniles, where L is body length.

In adult fish, steady subcarangiform swimming is only used at speeds of 3.6–5.4 Ls^{-1} (tail-beat frequencies of 5.0–8.3 Hz). Intermediate speeds involve unsteady swimming. In contrast, juvenile fish employ subcarangiform swimming at a range of intermediate velocities between the maximum labriform and burst speeds (2.3–8.4 Ls^{-1} at tail-beat frequencies of 4.0–12.5 Hz). These differences in swimming behaviour are discussed in relation to changes in life-style and muscle fibre type composition between juvenile and adult fish.

Burst swimming speeds in *N. neglecta* have been compared with equivalent data from temperate species. It seems likely that low temperature limits swimming performance in Antarctic fish. This is more noticeable in juvenile stages, which have much higher tail-beat frequencies than adult fish.

Chapter 4. The composition and distribution of mitochondria in the slow muscle of antarctic fish.

An ultrastructural study was carried out on the slow muscle fibres of five species of antarctic fish. The morphology of the capillary bed, muscle fibre composition and distribution and structure of mitochondria were determined using stereological techniques.

Estimates of cristae surface density within mitochondria (S_v(cr,mi) were significantly ($P \leq 0.05$) lower

in haemoglobinless/myoglobinless channichthyid fish (25.2-28.2$\mu m^2 \mu m^{-3}$) than in red-blooded species (32.2-37.0$\mu m^2 \mu m^{-3}$). Lower values of S_v(cr,mi) in channichthyid species suggest lower enzyme concentrations, oxygen consumption and ATP production capacity per unit volume of mitochondria than in red-blooded species.

Mitochondrial distribution was determined across the slow fibres of *Chaenocephalus aceratus*, *Notothenia gibberifrons* and the temperate species, *Callionymus lyra*. A lower decline in mitochondrial volume density towards the fibre centre was observed in the two antarctic species, especially *C. aceratus*. The homogeneous distribution and relatively high volume densities of mitochondria in antarctic fish suggests that oxygen diffusion between capillaries and mitochondria is not a limitation. High values of intermyofibrillar mitochondrial volume density (0.51-0.25) may be related to reduced diffusion distances between mitochondria and myofibrils. Shorter diffusion pathlengths may compensate for limitations to diffusion induced by low temperatures.

Chapter 5. Scaling effects on the neuromuscular system, twitch kinetics and morphometrics of the cod, *Gadus morhua*.

Twitch contraction time (time to 90% relaxation) in cod was relatively independent of total length, scaling in proportion to $L^{0.29}$. However, the extent to which contraction time determines swimming performance is not clear.

Mean cross sectional area and weight of the myotomal muscle scale geometrically with total length ($\alpha\ L^{2.05}$ and $L^{3.08}$ respectively). The geometric scaling of myotomal muscle mass is in accordance with previous estimates of the scaling of muscle power output (αL^3). However, there appears to be a lack of correlation between scaling of myotomal muscle cross sectional area and force generation ($\alpha > L^2$). This result might be explained by changes in the intrinsic properties of the contractile proteins during growth.

The number of endplates per fibre increased with increasing fibre length, from around 10 on fibres 2mm in length (~ 10cm fish) to 20 on 10mm fibres (~40cm fish). However, mean spacing between endplates increased from around 0.25mm to 0.50mm. These results are discussed in relation to the functional significance of multiple innervation in teleost fast muscle fibres. In the light of studies that demonstrate that teleost fast muscle fibres are able to generate propagated action potentials, the functional significance of polyneuronal innervation remains obscure.

Chapter 6. General Discussion.

The discussion focuses on the merits and disadvantages realised during this research, of examining adaptation in reductionist or holistic terms. This involves an examination of the relevance of muscle metabolism, structure and contractile properties to swimming

performance. Some possibly profitable directions for future research are discussed.

A summary of the Antarctic fish mentioned in this study.

Species.	Habitat of adult.
Order Perciformes	
Sub-Order Notothenioidei	
Family Nototheniidae	
Pagothenia borchgrevinki (Boulenger)	cryopelagic
Pagothenia (=*Trematomus*) *hansoni* (Boulenger)	demersal
Notothenia gibberifrons (Boulenger)	demersal
Notothenia neglecta (Nybelin)	demersal
Notothenia rossii (Fischer)	demersal/pelagic
Family Channichthyidae	
Chaenocephalus aceratus (Lônnberg)	demersal
Chamsocephalus gunnari (Lônnberg)	pelagic
Pseudochaenichthys georgianus (Norman)	demersal/pelagic
Family Bathydraconidae	
Psilodraco breviceps (Norman)	demersal/pelagic
Family Harpagiferidae	
Artedidraco mirus (Lônnberg)	demersal

CHAPTER 1.

GENERAL INTRODUCTION.

Swimming in fish has been studied for a wide variety of reasons. Many studies have concentrated on the metabolic costs of swimming, particularly migratory movements, in relation to the energy budgets of fish (Brett & Glass, 1973; Priede & Holliday, 1980; Weatherley *et al.* 1982). Problems of flow obstruction due to the entrapment of fish in water intakes for electric generating stations, have also led to studies of fish swimming performance (Dorn *et al.* 1979; Turnpenny, 1983). Knowledge of the prolonged swimming speeds and endurance of fish allows prediction of their behaviour in the path of trawls. This may lead to more precise fishing techniques (Wardle, 1986). Locomotion in fish has also been of intense interest to hydrodynamicists. For instance, specialised body shapes of fast swimming scombroids have been extensively studied in relation to drag reduction mechanisms (Magnuson,1978). The effects of prolonged exercise (Davison & Goldspink, 1977; Davison, 1983) and sprint training (Gamperi *et al.* 1988) on growth is of relevance to fish handling and aquaculture.

This study examines fish swimming as a function of the structure and performance of the locomotor musculature. Of particular interest is the plasticity of muscle systems in relation to evolutionary adaptation and growth.

Of all environmental parameters which limit life, temperature is the most pervasive; hence adaptations with respect to temperature are multiple and affect every biological process. Part of this study is concentrated on the specialised physiology that has evolved in antarctic

fish in relation to the low environmental temperatures at which they live.

The way animals move and how these movements vary with size have long been of interest to biologists (see Pedley, 1977). This study will examine some of the consequences of fish growth to swimming performance and muscle function. Variations in the effects of temperature with growth will also be considered.

1.1. ADAPTATION AND TEMPERATURE.

The temperature range within which life occurs is wide. For instance, methanogenic bacteria live at high pressure in oceanic thermal vents, at temperatures in excess of 250° C (Baross & Deming, 1984). At the opposite end of the temperature scale, many invertebrates survive antarctic winters at temperatures as low as -60° C (Clarke, pers. comm.). Amongst fish, *Oreochromis alcalicus grahami* live in the highly alkaline geothermal springs around the shore of Lake Magadi, Kenya. Temperatures within these springs are in the region of 25-40° C (Reite *et al.* 1974). In contrast, antarctic fish inhabit the world's coldest marine environment. In McMurdo Sound (78° S) fish live year round at temperatures at which the seawater is in equilibrium with ice, at about -1.9° C (Littlepage, 1965). The species of fish used in this study were collected from either South Georgia (54° S) or Signy Island (61° S), where temperatures are slightly higher but fall below 0° C for a large part of the year (Everson, 1977; North & White, 1987).

The evolution of Antarctic fish.

During the Paleozoic and Mesozoic Eras Antartctica was joined with Australia, Africa, India and South America in the super continent of Godwanaland. The break up of Godwanaland and the continental drift of Antarctica to it's present polar position began about 60 million years ago, at the beginning of the Cenozoic Era (Kennet, 1977). As Antarctica migrated south it carried with it the ancestors of most of today's antarctic ichthyofauna.

The establishment of the circum-antarctic currents and a steep temperature gradient at the Antarctic Convergence, effectively isolated the aboriginal antarctic fish population. This isolation has resulted in a high degree of endemism in modern antarctic fish (Kennet, 1978). Of the 203 species (including some sub-species) recorded from the Antarctic region, 88% are endemic (Andriashev, 1987). Members of the suborber Notothenioidei constitute the basis of the antarctic fish fauna and make up 53% of the species. Within the Notothenioidei 97% of species are endemic to Antarctica (Andriashev, 1987).

Adaptation and Compensation.

Biological changes in response to altered environment can occur over three different time courses. They can involve immediate responses, acclimation or acclimatization occurring during days or weeks or genetic adaptations which may persist during many generations. This study will concentrate on long term adaptations in antarctic fish.

Adaptations can be categorised as either resistance or capacity adaptations (Prosser, 1987).

Resistance adaptations.

Resistance adaptations occur at environmental extremes and may allow an organism to live in conditions that are not optimal in terms of rate functions. There are numerous examples of resistance or tolerance adaptations that allow antarctic fish to withstand very low temperatures (Clarke, 1983; Macdonald *et al.* 1987). The presence of glycoprotein antifreezes in the body fluids of antarctic fish prevents the growth of ice crystals in the blood plasma at temperatures down to $-2.7°C$ (DeVries & Lin, 1977). Membranes and proteins have structures which are specialized for function at around $0°C$ (Cossins & Bowler, 1987). For example, brain tubulin subunits from antarctic fish assemble *in vitro* and remain stable at the low ambient temperatures experienced by these fish, whereas homologous proteins from homeotherms depolymerize at similar temperatures (Dietrich & Overton, 1986).

Capacity adaptations.

Capacity adaptaions involve the compensation of rate processes. Compensatory adaptations show similar patterns to those defined by Precht (1958) and Prosser (1973) for acclimatory responses (Fig. 1). The activities of a large number of critical metabolic enzymes exhibit complete or partial compensation to low temperatures in antarctic fish, including many muscle enzymes (Greene & Feeney, 1970;

Feeney & Osuga, 1976; Johnston *et al.* 1975, 1977; Johnston & Walesby, 1977, 1979; McArdle & Johnston, 1980). The activities of non-equilibrium enzymes provide information about potential carbon flux through metabolic pathways (Newsholme & Paul, 1983). Slow muscle activities of the non-equilibrium enzymes hexokinase (glucose oxidation) and carnitine palmitoyltransferase (fatty acid oxidation), are similar in notothenioids at 0° C to those in temperate fish at 15° C, exhibiting almost complete temperature compensation (Johnston & Harrison, 1985).

Adaptation to temperature of muscle contractile properties.

Cold-water fish exhibit many examples of resistance adaptations in proteins that are involved in the contractile process. An increased susceptibility to heat denaturation has been reported in parvalbumins (Hamoir & Gerardin-Othiers, 1979), myosin (Johnston *et al.* 1975; Persnowska & Smialowski, 1981), myofibrillar ATPase (Walesby & Johnston, 1979) and whole muscle fibres (Johnston, 1987). Sensitivity to heat may be a consequence of alterations in molecular structure, in order to improve low-temperature function. This section will briefly summarise capacity adaptations of the musculature of ectotherms (see Bennett, 1985; Johnston & Altringham, 1988a; for reviews). Particular emphasis will be placed on the specialised contractile properties of skinned muscle fibres from antarctic notothenioids.

Isometric and isotonic measurements of isolated muscle preparations are not directly applicable to locomotion.

However, they do provide an indication of the capacity of muscle systems to alter under environmental pressures. Skinned muscle fibres from antarctic fish, when fully activated, generate higher isometric tension (Po) at 0°C than similar fibres from temperate and tropical species at their preferred body temperatures (Johnston & Brill, 1984; Johnston & Altringham, 1985; Altringham & Johnston, 1986). Below the normal physiological range of each species tension generation is temperature dependent. At -5°C, fibres from *Notothenia neglecta* developed 2.4x the tension generated by the temperate *Platichthys flesus* and 14x that of the tropical *Oreochromis niloticus* (Altringham & Johnston, 1986). Skinned (demembranated) fibres are essentially a system of isolated myofibrils, with an energy supply under the control of the experimenter. ATP utilisation by fibres can be measured as an increase in the total ADP content of the post-contraction activating solution, by use of high performance liquid chromatography (Altringham & Johnston, 1985). This provides an indication of the "economy" (maximum isometric tension divided by ATP moles hydrolysed myosin $head^{-1}$ s^{-1}) intrinsic to the force generating system. At -5°C the relative cost of isometric contraction is in the ratio 1 : 2 :10 in *N. neglecta*, *P. flesus* and *O. niloticus*, respectively.

Higher economy in the muscle fibres of antarctic fish may be due to an increase in the force produced during the cross-bridge cycle. This could be achieved by an increase in the proportion of time the cross bridges spend in the force generating state of the cycle (Altringham & Johnston,

1986). An increase in time spent in the bound state would raise the number of cross bridges attached at any one time and therefore the force. Alternatively, the bound time may remain constant, instead more force could be generated by each cross bridge (Altringham & Johnston, 1986).

In contrast, the maximum cross bridge cycle times during shortening do not appear to have compensated to different temperatures. There appears to be no correlation between maximum isotonic contraction velocity and environmental temperature, even between antarctic and tropical species (Johnston & Brill, 1984; Johnston & Altringham, 1985). An alteration of the proportion of time in each state during the cross bridge cycle, may have evolved to compensate for differences in the temperature dependence of force generation, contraction velocity and "economy of contraction" (Altringham & Johnston, 1986).

The relationship between force and velocity, as defined by the force-velocity (P-V) curve, affects maximum mechanical output and the efficiency of work output (Woledge, 1968). The curvature of the P-V relationship can be defined by the constant "a" in Hill's hyperbolic (1938) equation. Alternatively, the contractile properties of certain live (electrically excitable) fibre preparations, are better described by a hyperbolic-linear curve (Marsh & Bennett, 1986). From this equation the ratio Wmax/Vmax.Po, where Wmax is maximum power output; is inversely related to the curvature of the P-V relationship. The less curvature in the relationship, the higher the velocity and therefore the power output for any given tension.

The shape of the force velocity curve is independent of temperature in the majority of live amphibian and reptilian muscle preparations (Lannergren, 1978; Marsh & Bennett, 1985; John-Alder & Bennett, 1987). For example, there is little variation in the power ratio used to describe the P-V relationship, in the fast glycolytic fibres of *Dipsosaurus dorsalis* at temperatures from 15-40° C (Marsh & Bennett, 1985). There is limited evidence that the P-V relationship may alter between fish from differing environmental temperatures. In skinned muscle fibre preparations the degree of curvature of the P-V relationship is higher in a tropical (*Makaira nigricans*) than an antarctic species (*Chaenocephalus aceratus*) (Altringham & Johnston, 1985).

However, more recent studies using live fibre preparations from fish, indicate that force-velocity relationships cannot be accurately defined from the contractile properties of skinned fibres (Curtin & Woledge, 1989; Altringham & Johnston, 1988a). Intraspecific variation in the P-V relationship with temperature, has been demonstrated in live preparations from the temperate *Myoxocephalus scorpius* (Langfeld *et al.* 1989). The curvature of the P-V relation decreases with a reduction in temperature from 8° C (Wmax/Vmax.Po = 0.126) to 1° C (Wmax/Vmax.Po = 0.136) in *M. scorpius*. This change in curvature between 8° C and 1° C represents an increase in relative maximum power output by about 15% (Langfeld *et al.* 1989).

Evidence from both live and skinned fibre preparations, would therefore suggest that some capacity exists in fish to alter muscle power output by changing the curvature of the P-V relation. However, the greatest contribution to increased power output at low temperatures is derived from capacity adaptations of maximum tension generation (Altringham & Johnston, 1985)

Embryonic development and lifestyle in antarctic fish.

At a more integrated level, temperature has a considerable influence on development, growth, metabolism and reproduction of fish. One measure of resistance adaptations in an organism is it's capacity to reproduce (Prosser, 1987). This section details several specialised aspects of the reproductive strategy of notothenioid fish. In the light of specialised lifestyle strategies, the controversy over the existence of metabolic cold adaptation will be discussed.

The rate of embryonic development within and between fish species is closely correlated to environmental temperature (Herzig & Winkler, 1986, Blaxter, 1988). Studies on ovary development in *Notothenia neglecta*, suggest that oogenisis in notothenioids generally may take 2 years (Everson, 1970). Prolonged oogenisis is linked to the production of large eggs and low fecundity in comparison to temperate water fish (Fig. 2) (North & White, 1987). The small eggs of many temperate marine species hatch within a few hours or days, producing, relatively small larvae (Russel, 1976). In comparison, notothenioid

larvae hatch months after spawning and are large, although not especially advanced (Fig. 2) (Twelves, 1970; Burchett, 1983; Efremenko, 1983; North & White, 1987). Initially, it would appear that the particularly slow development of notothenioids is a consequence of low environmental temperature.

Alternatively, relatively low fecundity, large egg size and slow development in antarctic species has been attributed to the evolution of K-strategy lifestyles rather than low temperatures (Clarke, 1980, 1983). The concept of the r-K continuum describes strategies in terms of the relative energetic investment in different phases of the lifestyle (Pianka, 1970). In contrast to r-selected species, K-selected species exhibit large size, delayed development and reproduction, higher adult survival and production of few young (Pianka, 1970). K-selected species are normally evolved to exploit limited environmental resource supplies.

The evolution of K-strategy lifestyles in antarctic marine poikilotherms is thought to be linked to the marked seasonality of primary production (Clarke, 1983, 1988). Of the few studies of year round growth or condition index carried out on antarctic fish, both *N. rossii* (Burchett, 1983) and *N. neglecta* (Everson, 1970) appear to restrict growth to a few summer months, when primary production is at a peak. It is argued (Clarke 1983) that an important consequence of the evolution of k-strategies is that it allows a low basal metabolic rate in polar organisms. Although the strategy of prolonged development, slow growth

and low basal metabolic rates may be common amongst antarctic invertebrates, there is some controversy regarding the metabolism of polar fish.

Metabolic Cold Adaptation.

The concept of metabolic cold adaptation implies that the resting metabolic rate of a polar fish would be higher than that of a warmer water fish acclimated to the same low temperature (Krogh, 1916). Early measurements of metabolic rate in polar fish advanced the theory of metabolic cold adaptation (Scholander, 1953; Wohlshlag, 1960, 1964).

The appreciation of the effects of stress, hypoxia and nutritional status have led to some doubt in early measurements of elevated basal metabolic rate and therefore metabolic cold adaptation (Holeton, 1970; Clarke, 1980, 1983). In addition, difficulties arise in directly comparing metabolic rates between temperate and antarctic species. Antarctic fish are highly stenothermic (Somero & DeVries, 1967) and eurythermal temperate fish cannot be acclimated to freezing temperatures. Therefore, comparison of metabolic rates requires extrapolation of values for temperate fish to antarctic temperatures.

Studies taking account of these problems have demonstrated large interspecific variations but continue to indicate that some degree of metabolic cold adaptation exists (Hemmingsen & Douglas, 1977; Wells *et al*, 1984; Wells, 1987). In addition, mesopelagic fish from antarctic waters at -0.5°C have similar metabolic rates (not resting

rates) to those of mesopelagic fish caught off the coast of California at 5° C (Torres & Somero, 1988).

There are several suggestions for the possible functional significance of metabolic cold adaptation. The maintenance of high concentrations of antifreeze glycoprotein may require elevated resting metabolic rates (Macdonald *et al.* 1987). There is evidence that increased scope for activity is coupled to high resting metabolic rates in trout, *Salmo gairdneri* (Weiser, 1985). Elevated basal metabolic rates in antarctic fish may also permit higher scopes for activity. In addition, there are suggestions that low temperatures affect the balance between the passive diffusion of ions through transmembrane channels and the active transport of ions against concentration gradients. Antarctic fish may require high resting metabolic rates to maintain the balance between ion diffusion and active transport (Hochachka, 1988).

1.2. ASPECTS OF SCALING IN FISH LOCOMOTION.

In addition to environmental temperature, growth in fish has a profound effect on the muscular system, swimming performance and behaviour of fish (for reviews see Wardle, 1977; Webb, 1977; Weihs, 1977). In order to discuss the relationship between swimming performance and size and/or temperature, a brief description of various relevant aspects of fish locomotion are required.

Swimming in fish: modes of locomotion.

Fish swim in a variety of modes, involving both the action of undulatory body waves and/or the use of median or paired fins. The propulsive movements of fish were first classified by Breder (1926) and have been recently reviewed by Webb (1975) and Blake (1983). Swimming modes involving the action of body waves range from anguilliform (as seen in eels) to thunniform (as seen in scombroids). Classification is dependant on the specific body wavelength and the proportion of the body that undulates. This study is largely concerned with fish that swim in a subcarangiform mode, with less than one and more than one half a wavelength on the body at any one time. Subcarangiform motion is typical of pelagic, fusiform teleosts such as salmonids, gadoids and cyprinids.

Common to many subcarangiform swimmers, especially at low speeds (Brett & Sutherland, 1965; Webb, 1973; Videler, 1981), is the use of labriform motion. Labriform motion is powered by oscillatory movements of the pectoral fins. The detailed kinematics and speeds at which labriform motion is employed differ between species; as described in the angelfish, *Pterophyllum eimekei* (Blake, 1980) and in the shiner seaperch, *Cymatogaster aggregata* (Webb, 1973).

Activity levels of swimming in fish.

Three levels of swimming have been extensively used in the discussion of locomotion in fish (Brett, 1965; Webb, 1975; Wu & Yates, 1978; Blake 1983). The division of swimming activity into three zones is based upon the

velocity-time curves (fatigue curves) described for sockeye salmon, *Oncorhynchus nerka* (Fig. 3) (Brett, 1964, 1965; Brett & Glass, 1973). These activity levels are thought to represent physiologically relevant speeds. Sustained motion could be defined as swimming powered purely by aerobic metabolism, prolonged activity by both aerobic and anaerobic contributions and burst activity is powered anaerobically (Blake, 1983). In practise, distinction between activity levels is not clear and large variations exist between the endurance of different species (Fig 4). Interspecific variations between species requires that in order to simplify matters, sustained motion will be used to describe all steady swimming speeds other than short duration bursts.

Scaling and fish morphology.

Many morphological and physiological variables (y) can be scaled relative to body size (X), according to allometric equations of the general form

$$y = a \cdot X^b$$

$$\log y = \log a + b \cdot \log X$$

i.e. when the two variables are plotted on logarithmic co-ordinates, the result is a straight line. Body weight (M_b) is commonly employed as a measure of animal size in scaling problems. Total body length (L) is more relevant to the hydrodynamics of swimming and is therefore generally used to discuss scaling and fish locomotion. The exponent of an allometric equation indicates the appropriate model

describing a scaling relationship (for reviews see Schmidt-Nielsen, 1984; McMahon, 1973).

Almost by definition growth of fish larvae is allometric. Highest growth rates occur in caudal and rostral regions (Fuiman, 1983). Fast growth in the caudal region increases the surface area available for propulsion and the amount of musculature. Beyond the larval stage, as fish grow morphometric parameters appear to scale geometrically. For example, the surface areas of sockeye salmon, (*Oncorhynchus nerka*) and rainbow trout (*Salmo gairdneri*), scale in proportion to $L^{2.14}$ and $L^{2.11}$, respectively (Webb, 1977). Body weight in fish also scales geometrically with L^3; a fact made use of by fisheries biologists in the von Bertalannfy equation (Cushing, 1975).

<u>Size and the hydrodynamics of swimming in fish.</u>

Due to the difficulties involved in directly measuring fish swimming movements, dimensional and hydrodynamic models have been widely used to estimate the effects of size on performance (Wardle, 1977; Webb, 1977; Weihs, 1977; Blake, 1983).

To swim at a steady forward velocity a fish must produce thrust equal to the drag resistance. The drag experienced by a swimming fish can be written as

$$\text{drag} = \tfrac{1}{2}\, \delta . S . U^2 . C_D . K\ (\cos \phi)$$

where δ = density, S = wetted surface area, U = swimming speed, C_D = drag coefficient for an equivalent flat plate, K = modifier term for drag increments of a self propelling flexing body and ϕ = angle subtended by the tail to the

direction of motion (Lighthill, 1977; Webb, 1975; Johnsrude & Webb, 1985). The geometric scaling of surface area ($S \alpha L^2$), has often led to the conclusion that drag is also proportional to L^2 and that the drag coefficient is a constant. However, the drag coefficient alters with size and is proportional to Reynolds Number (R_e), which is directly dependant on fish total length (L) and swimming speed (U)

$$R_e = L.U/v$$

where v = kinematic viscosity (Webb, 1975).

Boundary layer flow characteristics are dependant on the Reynolds Number. When Reynolds Number is low, viscous forces dominate and flow is laminar. At high Reynolds Number, viscous forces become less important as pressure changes associated with inertial forces create turbulence.

Amongst fish, Reynolds Number can vary from in the region of 10-200 for larval fish (Blake, 1983) to $>10^7$ for large, fast swimming scombroids (Magnuson, 1978). Changes in drag associated with increased Reynolds Numbers are thought to become critical at certain sizes and swimming speeds. For instance, using theoretical models Weihs (1980) has suggested that larval fish would alter their swimming style as they grow. In smaller larvae with lower Reynolds Numbers, it is more efficient to swim continuously due to the predominance of viscous forces. As size increases, inertial effects become more important and intermittent swimming, involving burst-coast type behaviour becomes more common (Weihs, 1980; Webb & Weihs, 1986). Development of dorsal and caudal fins and an alteration in swimming style

from anguilliform to subcarangiform in herring larvae, is possibly associated with the change in drag components (Batty, 1984).

Wardle (1977) has suggested that critical Reynolds Numbers limit maximum sustained swimming speeds. The high sustainable speeds observed in scombroids are due to their streamlined shape, which maintains laminar flow at higher speeds and higher Reynolds Numbers than on typical teleosts (Wardle, 1977).

Scaling, sustainable swimming and transport costs.

Critical levels of performance have often been used to discuss scaling problems; as they are believed to represent physiologically similar speeds for all sizes (Heglund *et al.* 1974; Hegland *et al.* 1982; Brett, 1965; Brett & Glass, 1973; Webb *et al.* 1984). For instance, Brett (1965) chose the maximum swimming speed that could be sustained for 60 min ($60_{min}U_{crit}$), as a representative critical performance level for sockeye salmon, *Onchorhynchus nerka*. Reduction of length specific critical swimming speed is scaled to $L^{0.63}$, in *O. nerka* (Brett & Glass, 1973). In addition, the metabolic scope for activity (oxygen consumption at $60_{min}U_{crit}$-standard oxygen consumption) alters with length to the factor $L^{3.02}$ (Brett & Glass, 1973). If the rate of oxygen consumption is divided by swimming speed the metabolic cost of transport can be estimated (Schmidt-Nielsen, 1984). The cost of swimming for *O. nerka*, at 3/4 $60_{min}U_{crit}$ is proportional to $M_b^{-0.25}$, suggesting that the

cost of transport per 1g is cheaper in larger fish (Schmidt-Nielsen, 1984).

An alternative estimate of the cost of transport involves both hydrodynamic models and measurements of metabolism (Webb, 1977). The ratio of thrust power output, estimated from detailed kinematics and hydrodynamic models (Lighthill, 1970) and aerobic power output, estimated from metabolic scope, gives an indication of aerobic efficiency (Webb *et al.* 1984). Aerobic efficiency in *S. gairdneri*, increases with length (Webb *et al.* 1984). In contrast, Froude efficiency (or the propeller efficiency), the ratio of thrust power to the mean rate of working, is independent of size. The increase in aerobic efficiency with length, whilst Froude efficiency remains scale independent, suggests an increase in muscle efficiency with size in fish (Webb *et al.* 1984).

Muscular efficiency in terrestrial locomotion has also been demonstrated to increase with size (Heglund *et al.* 1982). Slower stride frequency at critical reference speeds, decreases the cross-bridge cycling rate for larger animals, possibly allowing greater efficiency (Heglund *et al.* 1982). Smaller animals move proportionally faster at critical speeds, requiring faster frequency strides and therefore may have to recruit faster, less efficient muscle fibre types. A higher proportion of fast fibres have been found in the muscles of smaller birds and mammals (Close, 1972).

Histological and histochemical studies have not clearly indicated higher proportions of fast fibres in

smaller fish (Greer-Walker, 1970; Greer-Walker & Pull, 1975; Johnston & Camm, 1986; Dunn *et al.* 1989). Variations in swimming behaviour and activity alter fibre type proportions, possibly masking any scaling effect. However, analysis of the myofibrillar ATPase activity of fast muscle samples from a number of marine teleosts, showed a decline with length (Witthames & Greer-Walker, 1982). This would suggests that muscle fibres in smaller fish are faster, due to the relationship between myosin ATPase and maximum speed of contraction of muscle (Bárány, 1967).

Maximum swimming performance and size.

Reported burst swimming speeds range from 6 cms^{-1} in larvae of *Engraulis mordax* (Webb & Corolla, 1981), to 2134 cms^{-1} in *Acanthocybium solandri* (Walters & Fierstein, 1964). However, the lack of a conceptual framework and technical difficulties involved in measuring maximum performance, result in a limited amount of comparable data. In consequence, attempts have been made to estimate burst swimming speeds from theoretical models (Bainbridge, 1961; Wardle, 1977) and from muscle preparations (Wardle, 1975; Brill, 1978; Johnsrude & Webb, 1985; McVean & Montgomery, 1985; Webb & Johnsrude, 1988).

Slower twitch contraction times of isolated muscle blocks in larger fish, suggest decreased tail-beat frequency and maximum length specific speed with size (Wardle, 1975). The complications involved in extrapolating contractile properties of muscle to *in vivo* performance will be discussed in depth in subsequent chapters.

Difficulties arise in part, because of the complex fibre geometry within myotomes (Alexander, 1969; Wainwright, 1983).

Wardle & He (1988) have pooled data on swimming performance of many species from the literature. They have shown maximum burst swimming speeds to be proportional to $L^{0.80}$. When only temperate species are included, and data is restricted to measurements that are acceptable estimates of maximum speeds; maximum swimming speed is proportional to $L^{0.85}$ (Fig 5).

Escape reactions have been studied in detail in different sized *S. gairdneri* (Webb, 1984). The duration of the three stages that make up the fast-start reaction, increased with length of fish. In contrast, actual acceleration rates, velocity attained and distance covered in a given time, were length independent. The independence of acceleration on size, suggests that the outcome of predator-prey interactions would be determined primarily on reaction times of individuals, rather than by muscle performance (Webb & Johnsrude, 1988).

Figure 1.1.

Diagram of the possible patterns of compensation via capacity adaptation, of a biological rate process. Derived from Pretch (1958).

1. Over compensation.
2. Complete compensation.
3. Partial compensation.
4. No compensation, a Q_{10} effect.
5. Inverse compensation.

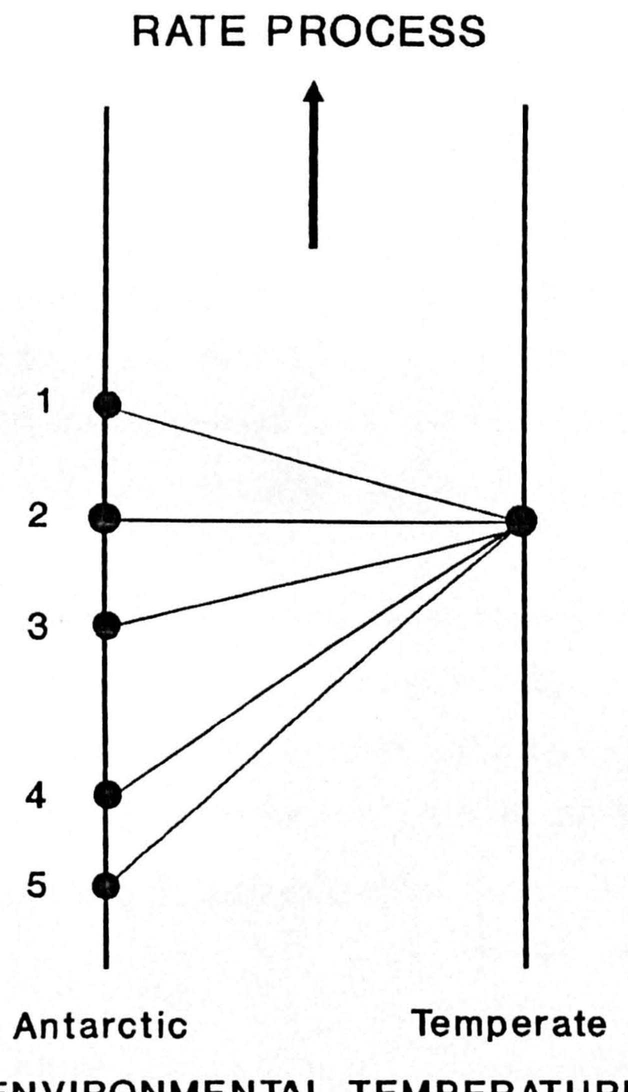

RATE PROCESS
1
2
3
4
5
Antarctic
Temperate
ENVIRONMENTAL TEMPERATURE

Figure 1.2.

Graph illustrating the range of egg diameter and larval length in temperate (solid lines) and antarctic (dashed lines) marine teleosts.

Temperate species: 1. *Engraulis mordax*; 2. *Scopthalamus maximus*; 3. *Gadus morhua*; 4. *Scomber scombrus*; 5. *Clupea harengus*; 6. *Pleuronectes platessa*; 7. *Hippoglosus hippoglossus* (for original data see Blaxter 1988).

Antarctic species: 1. *Notothenia nudifrons*; 2. *Harpagifer bispinis*; 3. *Notothenia neglecta*; 4. *Pagothenia hansoni*; 5. *Parachaenichthys georgianus*; 6. *Pseudochaenichthys georgianus*; 7. *Chaenocephalus aceratus*; 8. *Notothenia rossii* (for original data see North & White, 1987).

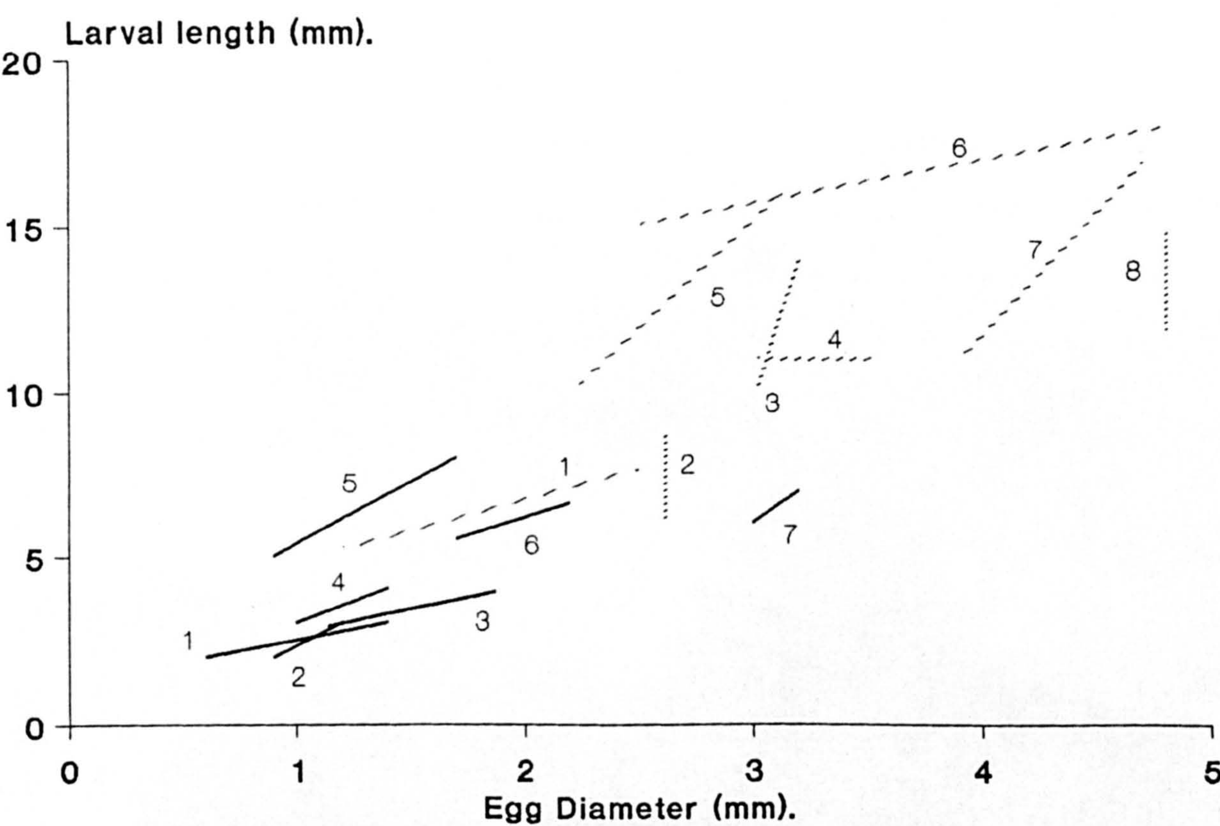
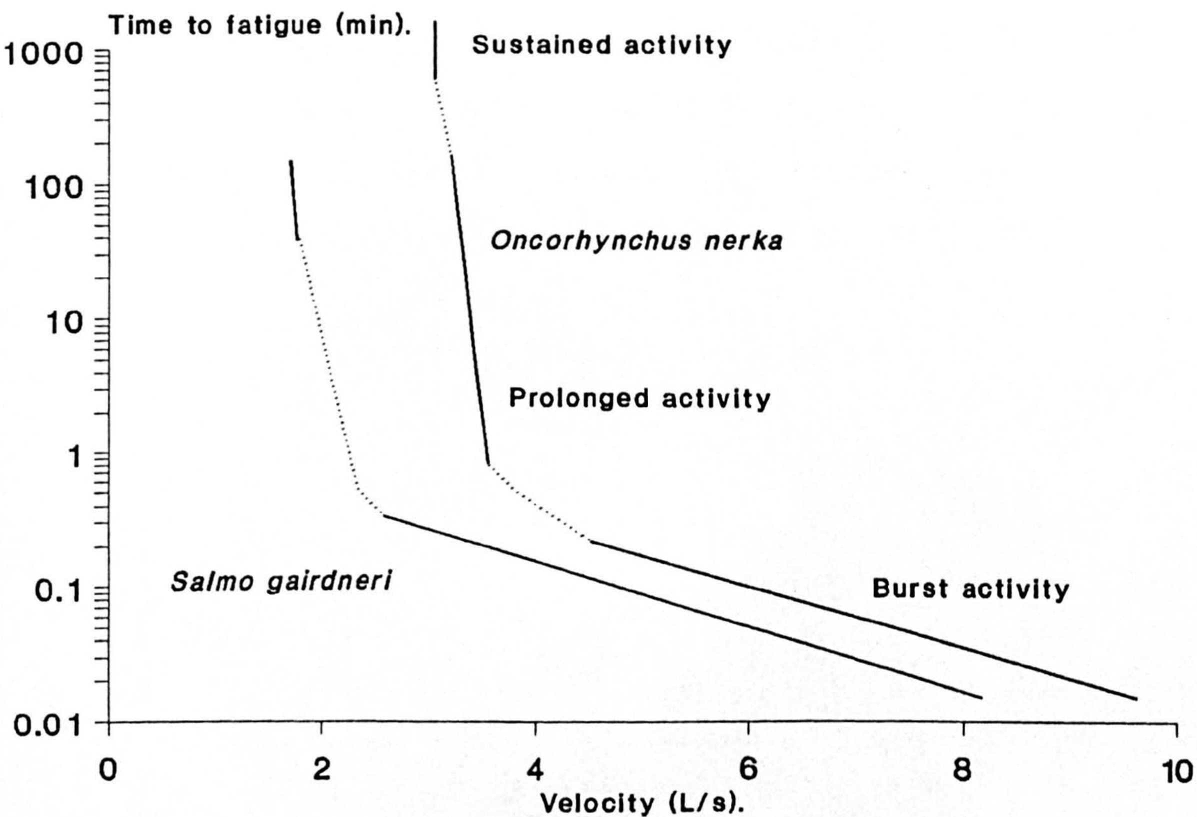

Larval length (mm).
20
15
10
5
0
0
1
2
3
4
5
Egg Diameter (mm).
1
2
3
4
5
6
7
8

Figure 1.3.

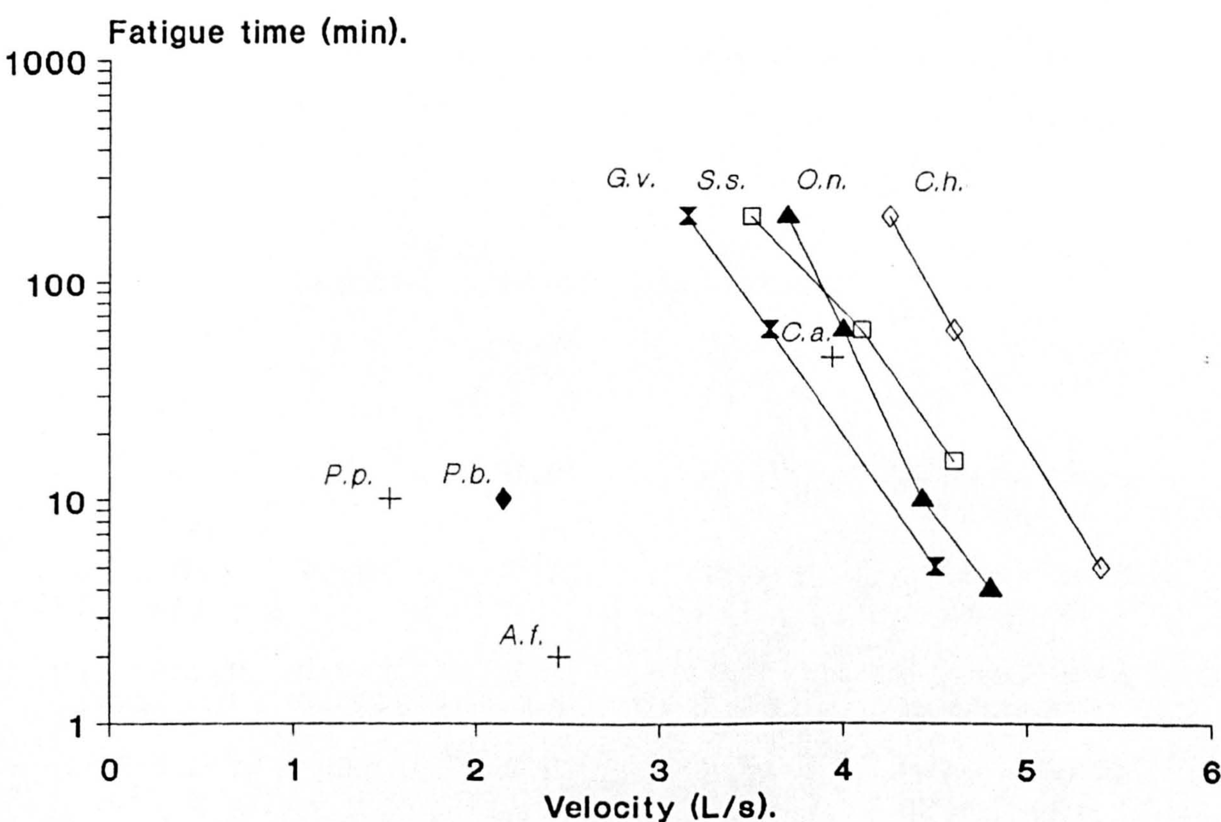
Fatigue time curves for juvenile sockeye salmon (*Oncorhynchus nerka*) and rainbow trout (*Salmo gairdneri*) as defined by Brett (1967).



Time to fatigue (min).
1000
100
10
1
0.1
0.01
Sustained activity
Oncorhynchus nerka
Prolonged activity
Salmo gairdneri
Burst activity
0
2
4
6
8
10
Velocity (L/s).

Figure 1.4.

Critical swimming speeds of various species of fish of 20-30cm in length. Source of data: *Oncorhynchus nerka* (Brett, 1964); *Lepomis gibbosus* (Brett & Sutherland, 1969); *Cymatogaster aggregata* (Webb, 1973); *Pleuronectes platessa* (Priede & Holliday, 1980); *Acipenser fulvescens* (Webb, 1986); *Clupea harengus*, *Gadus virens*, *Scomber scombrus* (He & Wardle, 1988); ♦ *Pagothenia borchgrevinki* (antarctic notothenioid, swimming at 0°C) (Forster *et al.* 1987).

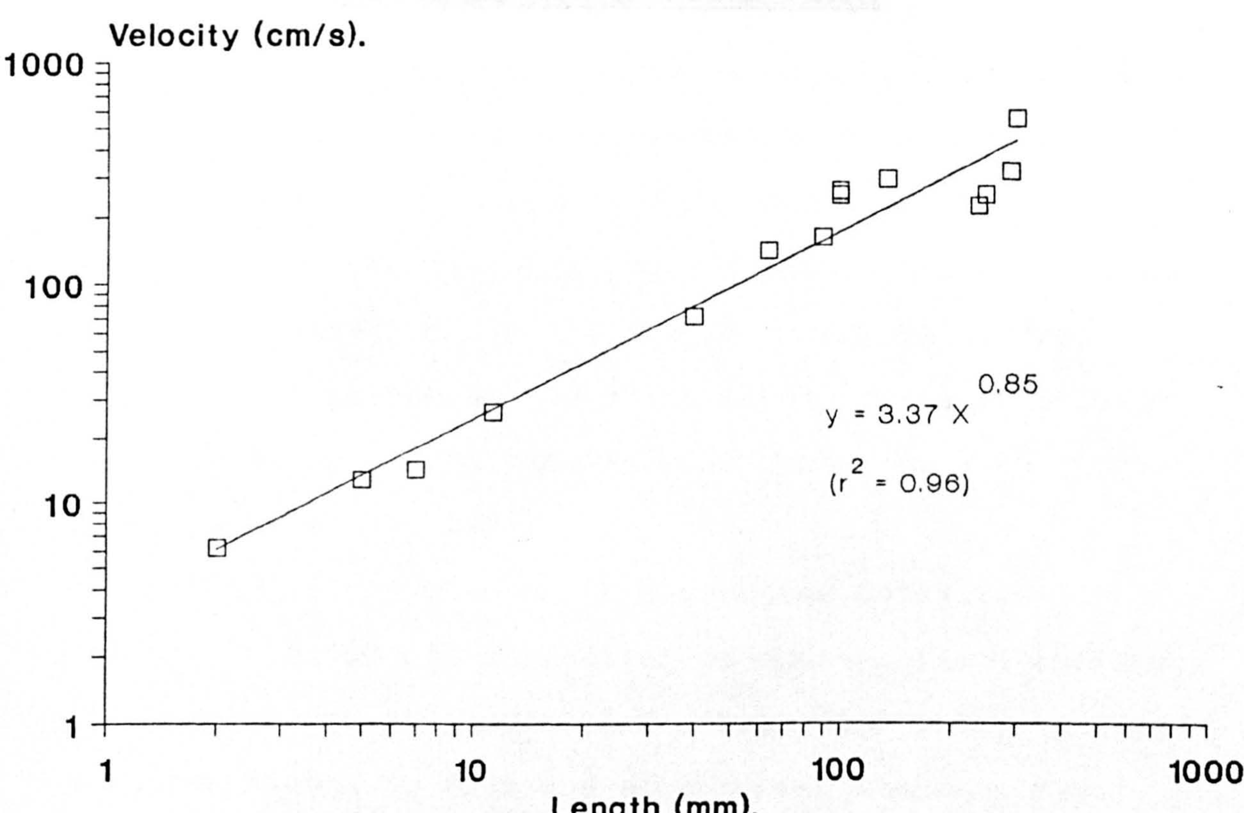


Fatigue time (min).
1000
100
10
1
0
1
2
3
4
5
6
Velocity (L/s).
G.v.
S.s.
O.n.
C.h.
C.a.
P.p.
P.b.
A.f.

Figure 1.5.

Allometric plot of the scaling relationship between burst swimming speed and length in temperate species. The data is listed below:

Species	Total length (cm)	Speed (L/s)	Speed (cm/s)	Source of data
Engraulis mordax	0.20	30.6	6.12	Webb &
	0.51	24.6	12.55	Corolla
	1.14	22.5	25.70	(1981)
Pleuronectes platessa	0.70	20.0	14.0	Batty (1981)
Trachurus symmetricus	6.4	21.7	138.9	Hunter & Zweifel (1971)
Leusicus leusicus	9.0	17.8	160.2	Bainbridge
	24.0	9.2	220.8	(1958)
Melanogramus aegelfinus	10.0	25.0	250	Wardle (1975)
Sprattus sprattus	10.0	26.0	260	"
Salmo salar	25.0	10.0	250	Wardle (1975)
Salmo gairdneri	4.0	17.5	70.0	Bainbridge
	13.5	13.0	292.5	(1958)
	29.3	10.8	316.0	
Scomber scombrus	30.5	18.0	550	Wardle & He (1988)


Velocity (cm/s).
1000
100
10
1
1
10
100
1000
Length (mm).
y = 3.37 X 0.85
(r2 = 0.96)

CHAPTER 2.

MUSCLE FIBRE TYPES AND METABOLISM IN POST-LARVAL AND ADULT STAGES OF NOTOTHENIOID FISH.

INTRODUCTION.

In the segmental muscles of fish two fibre types are commonly distinguished. Red fibres with an oxidative metabolism occur in the periphery, whilst the bulk of the myotome consists of white fibres with a glycolytic type of metabolism (Johnston *et al.* 1977; Newsholme *et al.* 1978; Zammit & Newsholme, 1979; Johnston & Moon, 1981; Johnston, 1981). In contrast to white fibres, red fibres are generally small in diameter, well vascularised and contain large numbers of mitochondria (Johnston, 1981). Detailed ultrastructural studies reveal differences in the extent of contact between the T-system and the sarcoplasmic reticulum (SR) in red and white fibres (Akster *et al* 1985). This variation in T-system/SR contact has been correlated with measured differences in tension development between fibre types (Akster *et al.* 1985)

Myofibrillar adenosine triphoshatase activities parallel the speed of contraction of muscle fibres (Bàràny, 1967). Histochemical studies have shown that white and red fibres correspond to fast and slow types, respectively (Johnston *et al.* 1974). In addition, the contractile properties of slow twitch and fast twitch myotomal fibres have been confirmed by both skinned (Altringham & Johnston, 1982) and live (Curtin & Woledge, 1987; Altringham & Johnston, 1988a) isolated fibre preparations.

The composition of fish myotomes is seldom limited to just two fibre types (for review see Bone, 1978). In some teleosts, a narrow band of fibres occurs between fast and slow types. These fibres have high titres of glycolytic

enzymes and an aerobic capacity and myofibrillar ATPase activity that is intermediate between slow and fast types (Johnston *et al.* 1974; Kryvi *et al.* 1981). In the dogfish *Scylorhinus canicula*, a superficial layer of myotomal fibres, which stain weakly for myofibrillar ATPase (Bone & Chubb, 1978), appear to have tonic contractile properties (Bone *et al.* 1986). Although their contractile properties have not been studied, similar fibers have been observed in several teleosts (Davison, 1983; Kilarski & Koslowska, 1983; te Kronnie *et al.* 1983).

The role of fast and slow myotomal fibres varies between species. Electromyographical studies demonstrate that in *Scylorhinus canicula* (Bone, 1966), *Clupea harengus* (Bone *et al.* 1978), *Morone saxatilis* (Sisson & Sidell, 1987) and *Katsuwonus pelamis* (Rayner & Keenan, 1967) only slow fibres are employed at sustainable swimming speeds. In other teleosts studied, fast fibre recruitment occurs at lower than maximum sustainable speeds (Hudson, 1973; Johnston *et al.* 1977; Bone *et al.* 1978, Rome *et al.* 1984). The correlation between contractile properties of fast and slow fibre types and their function at different swimming speeds, is discussed by Rome *et al.* (1988). The role of intermediate fibres has only been studied in *Cyprinus carpio*; the order of recruitment with increasing swimming speeds is slow > intermediate > fast (Johnston *et al.* 1977).

Information on swimming muscles in notothenioid fish is biased towards the adult stages of inshore demersal and cryopelagic species (Walesby & Johnston, 1980, Smialowska &

Kilarski, 1981; Davison & Macdonald, 1985; Dunn & Johnston, 1986; Harrison *et al.* 1987; Johnston & Camm, 1987). In both cases there is a distinct division between sustainable and burst swimming speeds reflecting the recruitment of pectoral fin and myotomal muscle respectively (Montgomery & Macdonald, 1984; Davison *et al.* 1988; Archer & Johnston, 1989). The well developed pectoral fin muscles are predominantly composed of slow twitch fibres, containing numerous mitochondria (Johnston & Harrison, 1985; Harrison *et al.* 1987; Johnston 1987). The myotomal muscles on the other hand are specialised for sprinting and are almost exclusively very large diameter fast twitch fibres, which rely on phosphocreatine (PCr) hydrolysis as an anaerobic energy supply pathway, limiting burst endurance (Dunn & Johnston, 1986). Sustained swimming speeds using the pectoral fins are relatively modest, ≤2 bodylengths/s (Montgomery & Macdonald, 1984; Forster *et al.* 1988; Archer & Johnston, 1989). Measurements of active metabolism suggest an aerobic scope of only 4-6 compared to 10-12 for athletic species such as trout or salmon (Bennett, 1978; Forster *et al.* 1988, Davison *et al.* 1988).

These studies have now been extended to a range of demersal-pelagic and pelagic fish, showing a wider range of activity patterns. All the species studied were collected during OBP Cruise 7 of the British Antarctic Survey to South Georgia during austral summer 1986-1987. Particular emphasis was given to the poorly studied post-larval stages of the life history.

MATERIALS AND METHODS.

Fish.

Sampling was carried out from the RRS John Biscoe II in the waters east of South Georgia (approximately 54° S, 36° 15' W) and within Cumberland East Bay.

Post-larval specimens were captured near the surface or in midwater using $1m^2$ or $8m^2$ rectangular midwater trawls. Demersal and demersal-pelagic species were caught at or near the bottom using an Agassis trawl.

Post-larval specimens sampled and their standard lengths (S.L.) included:

Artedidraco mirus Lönnberg (19-22mm S.L.),

Chamsocephalus gunnari Lönnberg (27-51mm S.L.),

Notothenia gibberifrons Boulenger (23-25mm S.L.),

Pagothenia (=Trematomus) hansoni (Boulenger) (30-35mm S.L.)

Pseudochaenichthys georgianus Norman (29-68mm S.L.).

Adult and juvenile fish sampled included:

Artedidraco mirus Lönnberrg (73-82mm S.L.),

Chamsocephalus gunnari Lönnberg (250-270mm S.L.),

Notothenia gibberifrons Boulenger (250-400mm S.L.),

Pagothenia (=Trematomus) hansoni Boulenger (344-376mm S.L.),

Pseudochaenichthys georgianus Norman (280-547mm S.L.),

Psilodraco breviceps Norman (94-162mm S.L.).

Tissue sampling and preparation.

Whole cross-sections of post-larvae were cut from a point immediately posterior to the anus. Samples of myotomal muscle from adults were dissected from a position approximately 2/3rd of the distance along the trunk (measured from the operculum). The pectoral fin adductor profundis muscle (m.ad.p) of adult fish was removed whole. Muscle blocks were mounted on cryostat chucks in an artificial embedding medium (OCT compound, Lab-Tek Products INC., USA). Tissue was rapidly frozen in 2-methylbutane cooled to near its freezing point (-160° C) by immersion in liquid nitrogen. Serial frozen sections were cut on a cryostat at -20° C and mounted on dry coverslips.

Histochemistry.

Succinic dehydrogenase (SDHase). Sections were stained for SDHase activity in a medium containing: sodium succinate, 80mM; phosphate buffer, 50mM; pH 7.4 (at 4° C), to which nitroblue tetrazolium was added to a concentration of 1mg/ml just prior to use (Nachlas *et al.* 1957).

α-Glycerophosphate dehydrogenase (α-GPDHase). Sections were incubated in the medium containing: D.L. α-glycerophosphate, 13.9 mM; Tris-HCl, 50mM; pH 7.6 (at 4° C) to which nitroblue tetrazolium was added to a concentration of 1mg/ml just prior to use (Wattenberg and Leong, 1960). (Incubation times in excess of 3 hours were required to develop significant staining for both SDHase and α-GPDHase).

Glycogen. Sections were incubated for 20mins in 1% periodic acid and stained with Schiffs reagent (PAS reaction).

Lipid. Staining was carried out using a Sudan Black B saturated solution in propyleneglycol for 30mins. Control sections were rinsed in acetone prior to staining.

Myofibrillar adenosine triphosphatase (ATPase). Sections were stained with or without preincubation at acid (1-10mins; pH 4.8 at 4°C) or alkaline pH's (0.5-1min; pH 10.4 at 4°C) using the buffers and methods described by Johnston *et al.* (1974).

Determination of proportion of different muscle fibre types.

The proportion of different muscle fibre types was determined from whole frozen cross-sections of post-larvae. For adult stages, frozen tissue was sectioned into steaks 2mm thick and stained for succinate dehydrogenase activity at 4°C. In both cases, the relative proportions of fibre types was determined using a camera lucida and digital planimetry.

Measurement of muscle fibre diameter.

Stained sections were used to identify fibre types. Once identified, fibres were drawn using the drawing arm of a microscope (Labophoto, Nikon, Japan) and their cross-sectional areas measured using a digital planimeter interfaced to a microcomputer (Hewlett Packard 86B). Muscle fibre diameters are derived from the diameters of a circle of identical area. Fifty fibres were measured at random for

each fibre type for each fish examined. In some cases fewer than 50 oxidative or small diameter fibres were present in each section, if so all fibres present were counted.

Assay of enzyme activities.

Muscle was dissected in the cold from freshly caught *Notothenia gibberifrons* and homogenised immediately. Fast muscle was dissected from approximately one third of the distance along the trunk, measuring from the operculum. Care was taken to ensure that the sample was deep enough to remove all traces of slow fibres. The m.ad.p. was removed intact and the deeper more pigmented layers were removed and stored on ice.

Tissues were minced with a scalpel, and homogenised in approximately 5 vols. of ice-cold buffer using a hand held sintered glass homogeniser. Homogenates were filtered through glass wool prior to assay of enzyme activities. The extraction buffer had the following composition: Tris HCL, 50mM; EDTA, 5mM; $MgCl_2$, 2mM; dithiothreitol, 1mM; pH 7.5. All enzymes were assayed spectrophotometrically at 2-3°C using a thermostat controlled cell holder. Substrate concentrations, co-factor concentrations and pH were set to yield optimal activities. The solutions and methods described in Dunn & Johnston (1986), were used to measure the following enzymes; adenylate kinase, creatine phosphokinase, pyruvate kinase, lactate dehydrogenase and malate dehydrogenase. The optimal pH for each enzyme was found to be pH 8.0.

Accurate tissue weights could not be obtained aboard ship. Approximate weights were obtained using a spring balance in order to standardise the dilutions. After homogenisation, 3ml aliquots of homogenate were removed, frozen (at -60° C) and returned to St. Andrews for subsequent analysis. The dry weight of tissue in 3ml of aliquot (the sample dilution factor) was obtained by subtracting the vial weight. Samples of rapidly frozen tissue were returned in liquid nitrogen and used for wet weight/dry weight analysis. This conversion factor was used to obtain enzyme activities expressed in terms of µmoles substrate utilised per g wet weight min^{-1}.

RESULTS.

Muscle histochemistry.

Although a wide range of preincubation and incubation times were attempted, it was not possible to identify different fibre types on the basis of their staining for myofibrillar ATPase activity. This confirms the findings reported for other antarctic fish species (Davison & Macdonald, 1985; Harrison *et al.* 1987). Instead slow fibres were identified on the basis of their intense staining reaction for SDHase and α-GPDHase (Fig. 5-13). Fast muscle fibres generally had larger diameters and stained more weakly for SDHase, α-GPDHase, PAS and Sudan Black B (lipid).

Post-larvae.

The distribution of muscle fibre types in the myotomes of post-larvae were similar in all the species examined (Fig. 1). Slow fibres form a superficial layer one fibre or two fibres thick (*A. mirus*), around the entire lateral surface of the trunk. A thicker layer of slow fibres occurs at the level of the lateral line nerve and at the major horizontal septa (Figs. 5 & 6). The remainder of the myotomal muscle is composed of fibres that stain weakly for SDHase, α-GPDHase, PAS and Sudan Black B. These fibres are highly variable in diameter and in all the species examined, the fibres bordering the myosepta stain somewhat more darkly for SDHase and α-GPDHase than does the bulk of the myotomal muscle. A population of very weakly staining fibres were found peripheral to the slow muscle at the lateral line (Figs. 1, 5 & 6).

Adult stages.

The distribution of fast and slow muscles in the myotomes of adult stages is shown in Fig. 2. There is greater interspecific variation in the relative proportion of fibre types than in the post-larval stages. The proportion of slow muscle ranges, in posterior segments, from 6%-9% in *C. gunnari* to 11%-22% in *P. breviceps*.

The pectoral fin adductor muscle of adult stages is largely composed of highly aerobic slow muscle fibres which stain intensely for SDHase, α-GPDHase and lipid (Figs. 11-13). There is a gradation of fibre types from narrow diameter, highly aerobic fibres to somewhat larger less

aerobic fibres in all the species examined. Much larger fibres are found in the deep part of the muscle and these probably correspond to the fast fibre types identified in the myotomes (Harrison *et al.* 1987). Generally the slow pectoral fibres stained more darkly for SDHase, α-GPDHase and Sudan Black B (lipid) than do slow fibres in the myotomes (Figs. 7, 8 & 10). Staining for glycogen (PAS) was more variable; highest intensity staining for PAS was observed in the slow myotomal muscle of *P. breviceps* (Fig. 9) and in the pectoral muscle of *P. hansoni*. Weak PAS reactions occurred in the slow muscle of the two haemoglobinless species, *P. georgianus* and *C. gunnari*.

Muscle fibre diameters.

The mean values and ranges of fibre diameters at post-larval and adult stages are shown in Tables 1 and 2 respectively. Frequencies of distribution of fibre size are shown in Figs. 3 and 4.

The mean diameter of the fibre types show no consistent differences in post-larval stages, in fact fast fibres have smaller mean diameters than slow fibres in *C. gunnari*, *N. gibberifrons* and *P. georgianus*. This contrasts with the situation in adults in which the diameter of fast fibres is substantially greater than that for slow fibres. The mean diameters of fast muscle fibres in the haemoglobinless species *C. gunnari* and *P. georgianus* are not larger than those found in species of similar size that contain haemoglobin.

Enzyme activities.

Enzyme activities in the muscle of *N. gibberifrons* are compared to those previously recorded in the muscle of *Notothenia neglecta* and *Chaenocephalus aceratus* (Table 3). Slow muscle fibres have significantly higher activities of pyruvate kinase and malate dehydrogenase than fast muscle fibres ($P<0.01$). Fast myotomal muscle in N. gibberifrons had relatively high activities of both glycolytic (LDH, PK) and immediate energy suppling enzymes (CPK, AK).

DISCUSSION.

Methods for staining myosin ATPase activity are not able to differentiate fast or slow fibre types in Antarctic fish (This report; Davison & Macdonald, 1985; Harrison *et al.* 1987). The contractile properties of histochemically identified fibre types have been determined in *Notothenia neglecta* (Johnston & Harrison, 1985). In view of these studies, muscle fibres have been described as fast and slow on the basis of their histochemical staining properties. Small diameter fibres with weak histochemical staining properties have been described in many teleosts. These fibres have a unique ultrastructural morphology suggesting that they are a distinct fibre type (Kilarski & Kozlowska, 1987). The small diameter fibres observed on the periphery of the myotomes in all notothenioid examined may have a postural or tonic role. They may hold the trunk in a fixed position during labriform locomotion and when stationary.

Post-larvae.

The myotomal muscles of post-larvae contain a superficial layer of fibres which stain intensely for oxidative enzymes (Figs. 1b, 5 & 6). This arrangement is similar to newly hatched larvae in other teleost species (Proctor *et al.* 1980; Batty, 1984; El-Fiky *et al.* 1987). Immunological studies with the tropical species *Brachydanio rerio* suggest that the myosin of these fibres is not identical to that of adult slow muscle (Van Raamsdonk *et al.* 1982). In the larvae of cyprinid fish the gills remain poorly developed for around 30 days such that most of the gas exchange probably occurs across the skin (El-Fiky *et al.* 1987). The development of the gill surface coincides with the remodelling of muscle fibres towards the adult type (El-Fiky *et al.* 1987). There is a similar change in the distribution of muscle fibre types in notothenioids between post-larval and adult stages. It seems likely that cutaneous gas exchange remains important in the pelagic juvenile stages.

The size of the post-larvae and the presence of well developed caudal and dorsal fins suggest a style of locomotion based upon inertial rather than viscous forces, as discussed for herring larvae by Batty (1984). This involves a characteristic search behaviour involving continuous or at least intermittent swimming (Weihs, 1980). It is known that post-larvae of *Champsocephalus gunnari*, *Pseudochaenichthys georgianus* and *Pagothenia hansoni* under take substantial diurnal vertical migrations, suggesting an active swimming behaviour (A. North, unpublished results).

Adult stages.

Notothenioids lack a swim bladder and are thought to derive from a benthic ancestor (DeWitt, 1971). One direction of evolution has been towards pelagism due to the rich source of food in the mid-water provided by krill (Nybelin, 1947; Andriashev, 1964). Adaptations to increase buoyancy include the accumulation of lipid, reductions in the mineralisation of skeleton and scales and modification of body shape (Eastman & DeVries, 1981, 1982, 1985). The reliance of demersal species on pectoral fin propulsion is maintained in cryopelagic species such as *Pagothenia borchgrevinki*, which spend long periods hovering in the water column (Montgomery & Macdonald, 1984). Studies of gill surface area suggest that some secondarily pelagic species, such as *Pleuragramma antarcticum*, are also relatively sluggish (Kunzmann, 1987). The adult stages of *C. gunnari* and *P. georgianus* (Channichthyidae) are known to migrate into mid-water to feed on krill (Permitin & Tarverdieva, 1972; Targett, 1981). The small proportions of slow muscle in the myotomes of these species suggest that they too rely on labriform locomotion and have relatively low maximum sustainable swimming speeds. In contrast, the most streamlined species studied, *P. breviceps*, has a well developed slow myotomal motor system (Figs. 2) *P. breviceps* is a relatively small demersal-pelagic species which probably undergoes frequent vertical migrations to feed on planktonic prey (Permitin, 1970; Andriashev, 1987).

Both mean and ultimate fibre diameter are higher in antarctic fish than values from ten freshwater temperate species studied by Weatherly et al. (1988). The maximum size of fibres attained in the temperate species ranged from 120–270µm. This is considerably smaller than in the antarctic species observed in this study. Myotomal fast fibres reached maximum diameters of between 199µm in *P. breviceps* and 499µm in *N. gibberifrons* (Table 2). Interestingly, largest fibres were found in the smaller temperate water fish (Weatherley et al. 1988); the reverse was observed amongst antarctic species in this study.

The particularly large size of fast fibres in notothenioids suggests that muscle growth is largely achieved through fast fibre hypertrophy. In addition, few fast fibres <80µm were observed in adult notothenioids (Fig 4), suggesting a low degree of hyperplasia in fish approaching maximum size. The size of fast fibres is a function of the growth rate of fish, large fibres from slow growth and small fibre from rapid growth (Weatherly & Gill, 1984). The large fibre size in notothenioids may be a reflection of their slow growth rates. Alternatively, the low surface to volume ratio of large diameter muscle fibres may serve to reduce the cost of maintaining ionic gradients (Smialowska & Kilarski, 1981).

There are two pathways for supplying energy for contraction in fast muscle, phosphagen hydrolysis and anaerobic glycolysis. Many more ATP equivalents are available from glycogen than phosphocreatine stores. The fastest method involving the production of ATP via the

hydrolysis of phosphocreatine is particularly important in typical "ambush-predators" such as *N. neglecta* (Dunn & Johnston, 1986) and *C. aceratus* (Johnston, 1987). Both of these species have relatively low activities of glycolytic enzymes in fast muscle. An inability to generate energy via anaerobic glycolysis for burst swimming has also been demonstrated in *P. borchgrevinki*, an active antarctic teleost (Davison *et al.* 1987). The high activities of glycolytic enzymes in the fast muscle of *N. gibberifrons* (Table 3) are consistent with it having a greater burst swimming endurance than either the "ambush predator" or the cryopelagic fish studied. *N. gibberifrons* has a sub-terminal mouth and feeds on benthos, especially infauna (Targett, 1981). Such interspecific differences in the energy metabolism of fast muscle probably reflect varied utilisation of burst swimming activity.

TABLE 2.1 Myotomal muscle fibre diameters in post-larval fish.

Species	Fish size (mm) (No. of fish)	Fibre type	n	Diameter (µm). (mean ± S.E.)	(range)
A. mirus	18–22 (4)	fast	274	37 ± 0.6	17–73
		slow	211	29 ± 0.4	15–51
		tonic?	232	16 ± 0.4	5–36
C. gunnari	29–51 (4)	fast	368	27 ± 0.5	16–76
		slow	209	32 ± 0.6	18–60
N. gibberifrons	23–25 (4)	fast	200	23 ± 0.5	8–39
		slow	200	29 ± 0.4	19–43
P. hansoni	30–35 (5)	fast	323	37 ± 0.6	14–76
		slow	210	33 ± 0.4	21–46
		tonic?	307	17 ± 0.5	5–62
P georgianus	29–58 (4)	fast	291	36 ± 0.9	9–75
		slow	269	41 ± 0.5	18–60
		tonic?	157	15 ± 0.3	5–27

TABLE 2.2. Muscle fibre diameters in adult fish.

Species	Fish size (mm) (No. of fish)	Fibre type	n	Diameter (μm). (mean±S.E.)	(range)
A. mirus	73-82 (3)	fast myotomal	142	122 ± 1.8	79-209
C. gunnari	248-270 (3)	fast myotomal	213	123 ± 2.4	38-368
		slow myotomal	295	72 ± 2.6	10-230
		m.ad.p. fast	150	117 ± 2.7	43-201
		m.ad.p. slow	304	55 ± 0.9	19-125
N. gibberifrons	250-400 (4)	fast myotomal	200	233 ± 6.4	57-499
		m.ad.p. slow	200	58 ± 1.3	25-104
P. breviceps	94-162 (4)	fast myotomal	200	138 ± 2.7	74-199
		slow myotomal	200	62 ± 1.9	23-115
		m.ad.p. slow	200	55 ± 1.2	20-110
P. georgianus	280-547 (3)	fast myotomal	150	196 ± 4.7	65-349
		slow myotomal	150	60 ± 2.0	17-135
		tonic myotomal	150	26 ± 0.7	9-52
		m.ad.p. fast	150	141 ± 3.2	59-258
		m.ad.p. slow	150	67 ± 2.5	23-190
P. hansoni	344-376 (3)	fast myotomal	205	214 ± 4.5	62-413
		slow myotomal	163	66 ± 3.4	11-217
		tonic myotomal	147	28 ± 0.7	6-47
		m.ad.p. fast	123	147 ± 2.7	50-299
		m.ad.p. slow	155	65 ± 1.6	36-125

TABLE 2.3. Muscle enzyme activities from antarctic fish.

Enzyme	*N. gibberifrons*		*N. neglecta*[a]	*C. aceratus*[b]	
	m.ad.p. slow	fast myotomal	fast myotomal	m.ad.p slow	fast myotomal
LDH	190 ± 17 (5)	400 ± 67 (5)	60	115	70
PK	48.1 ± 10.6 (8)	17.2 ± 2.6 (8)	5.8	10	29
CPK	401 ± 84 (5)	731 ± 134 (5)	474	825	112
AK	99.5 ± 19.6 (5)	103 ± 13 (5)	119	72	28
MDH	182 ± 40 (8)	14.5 ± 3.9 (8)			

Enzymes: LDH lactate dehydrogenase; PK pyruvate kinase; CPK creatine phosphokinase; AK adenylate kinase; MDH malate dehydrogenase.

Units: µmol substrate utilised g^{-1} wet weight min^{-1}. Values represent mean ± S.E. (sample size).

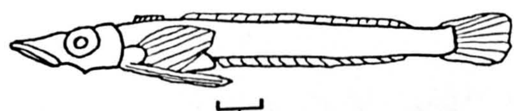
Previously published data from: [a] Dunn & Johnston, (1986);

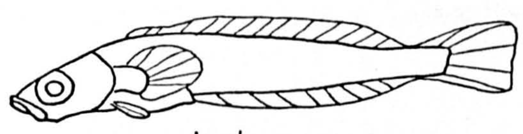
[b] Johnston, (1987).

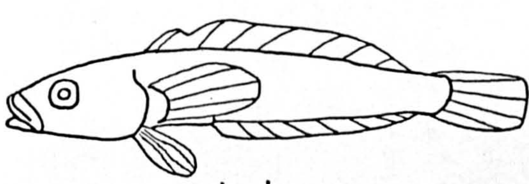
Figure 2.1.

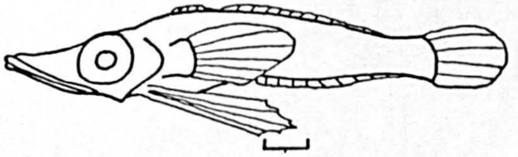
(a) & (b) Distribution of mytomal muscle fibre types in post-larvae. (a) Diagrams of lateral body shape of post-larvae. Values correspond to cross-sectional area of slow muscle as a percentage of total myotomal muscle cross-sectional area, at positions indicated. (bars = 2mm) ± S.D. (number of fish sampled = 4). (b) Diagrams of myotomal cross-sections of *A. mirus* and *P. hansoni*, illustrating the peripheral distribution of slow muscle.

a)

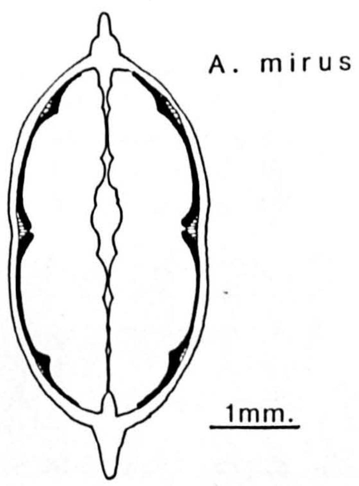








b)



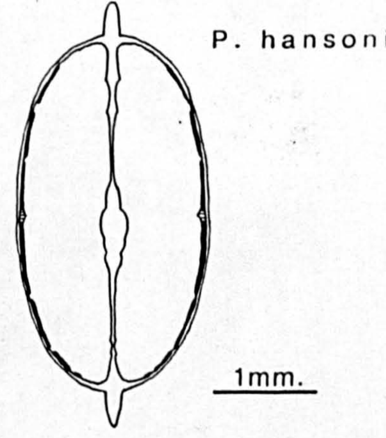


Figure 2.2.

Distribution of myotomal muscle fibre types in adults. Lateral body shapes (standard length mm) and diagrams showing distribution of fast and slow (in black) fibres, in cross-sections of the trunk. Values correspond to slow muscle cross-sectional area as a percentage of total muscle cross-sectional area (number of fish sampled = 1).

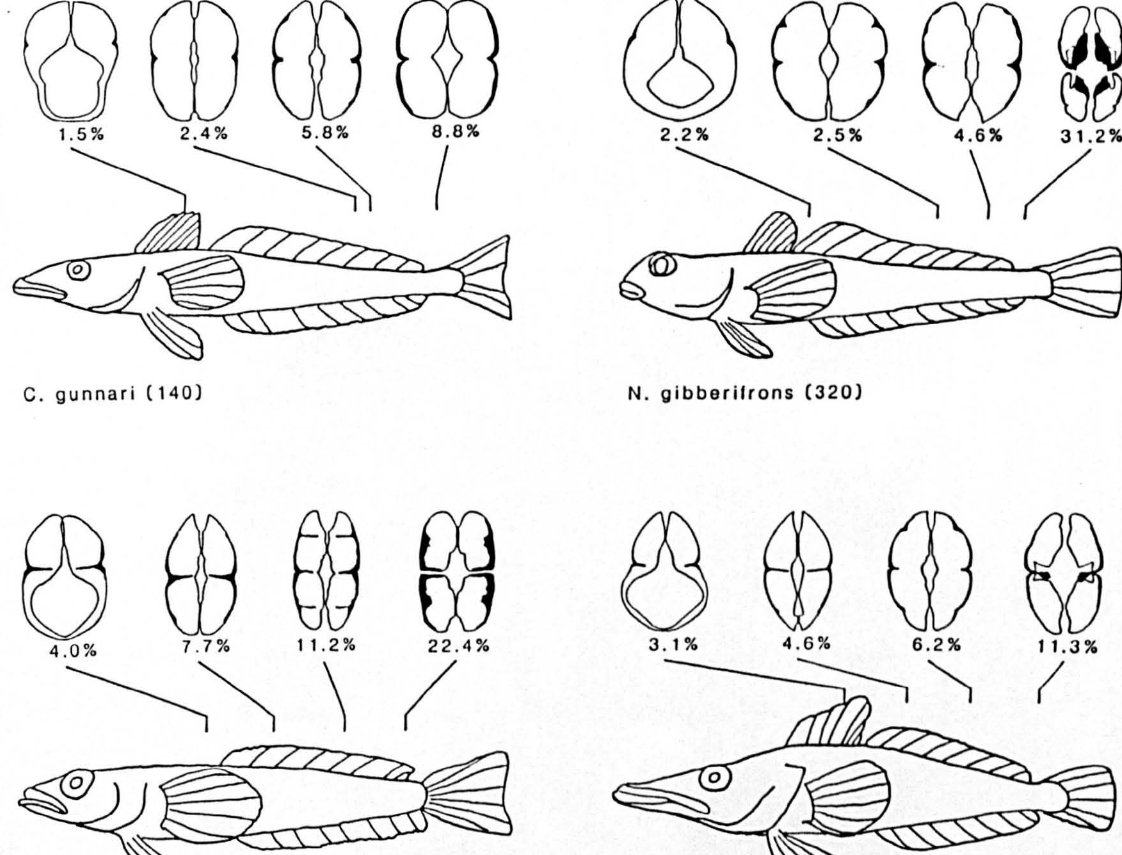
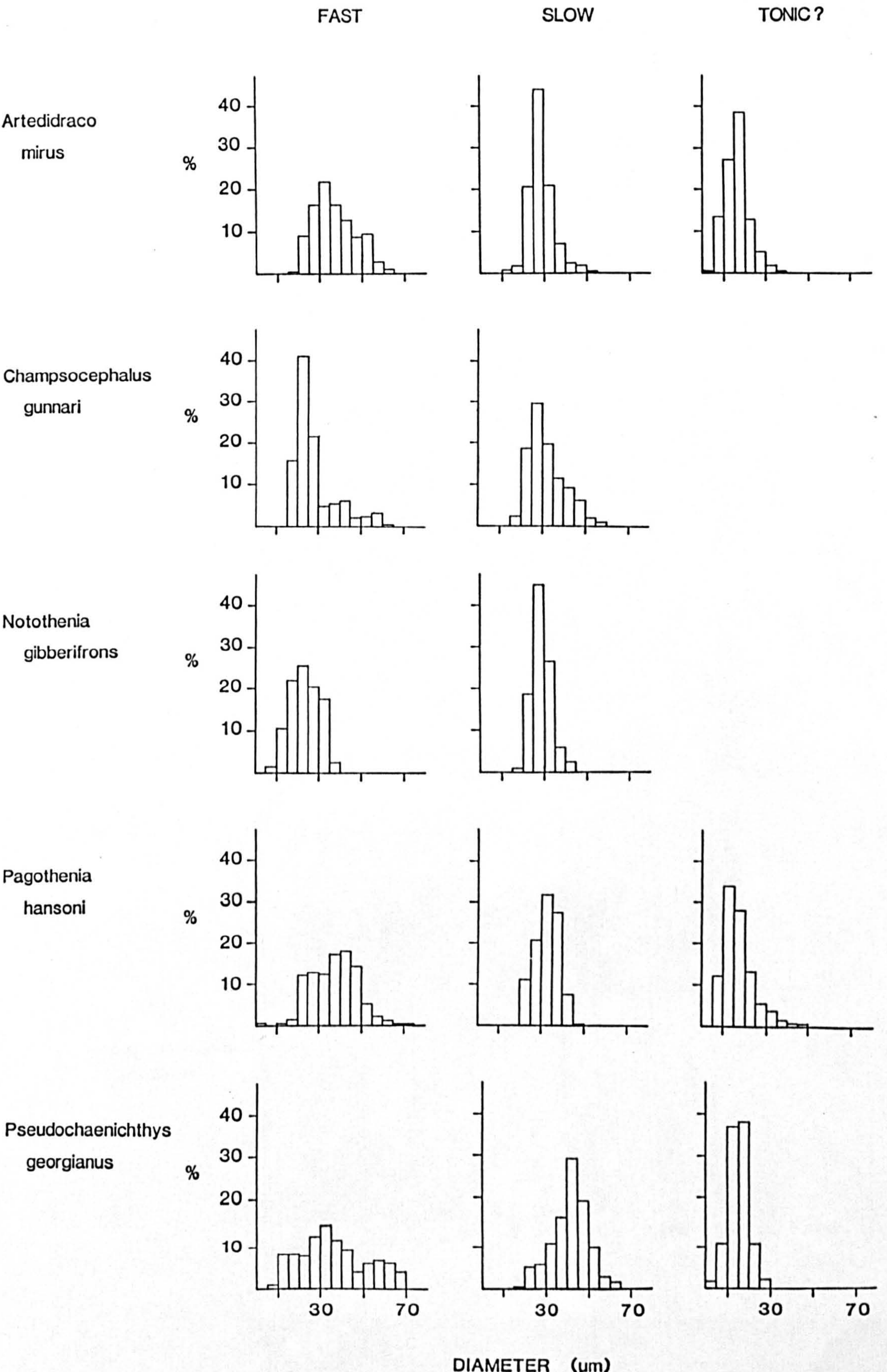

1.5%
2.4%
5.8%
8.8%
C. gunnari (140)
2.2%
2.5%
4.6%
31.2%
N. gibberifrons (320)
4.0%
7.7%
11.2%
22.4%
P. breviceps (112)
3.1%
4.6%
6.2%
11.3%
P. georgianus (170)

Figure. 2.3. Frequency distributions of the diameters of myotomal muscle fibres in post-larvae. Fish standard lengths, sample size and mean muscle fibre diameters are given in Table 2.1.



MYOTOMAL MUSCLE FIBRES
FAST
SLOW
TONIC ?
Artedidraco mirus
Champsocephalus gunnari
Notothenia gibberifrons
Pagothenia hansoni
Pseudochaenichthys georgianus
%
40
30
20
10
30
70
DIAMETER (µm)

Figure 2.4. Frequency distributions of the diameters of muscle fibres in adult fish. Fish standard lengths, sample size and mean muscle fibre diameters are given in Table 2.2.

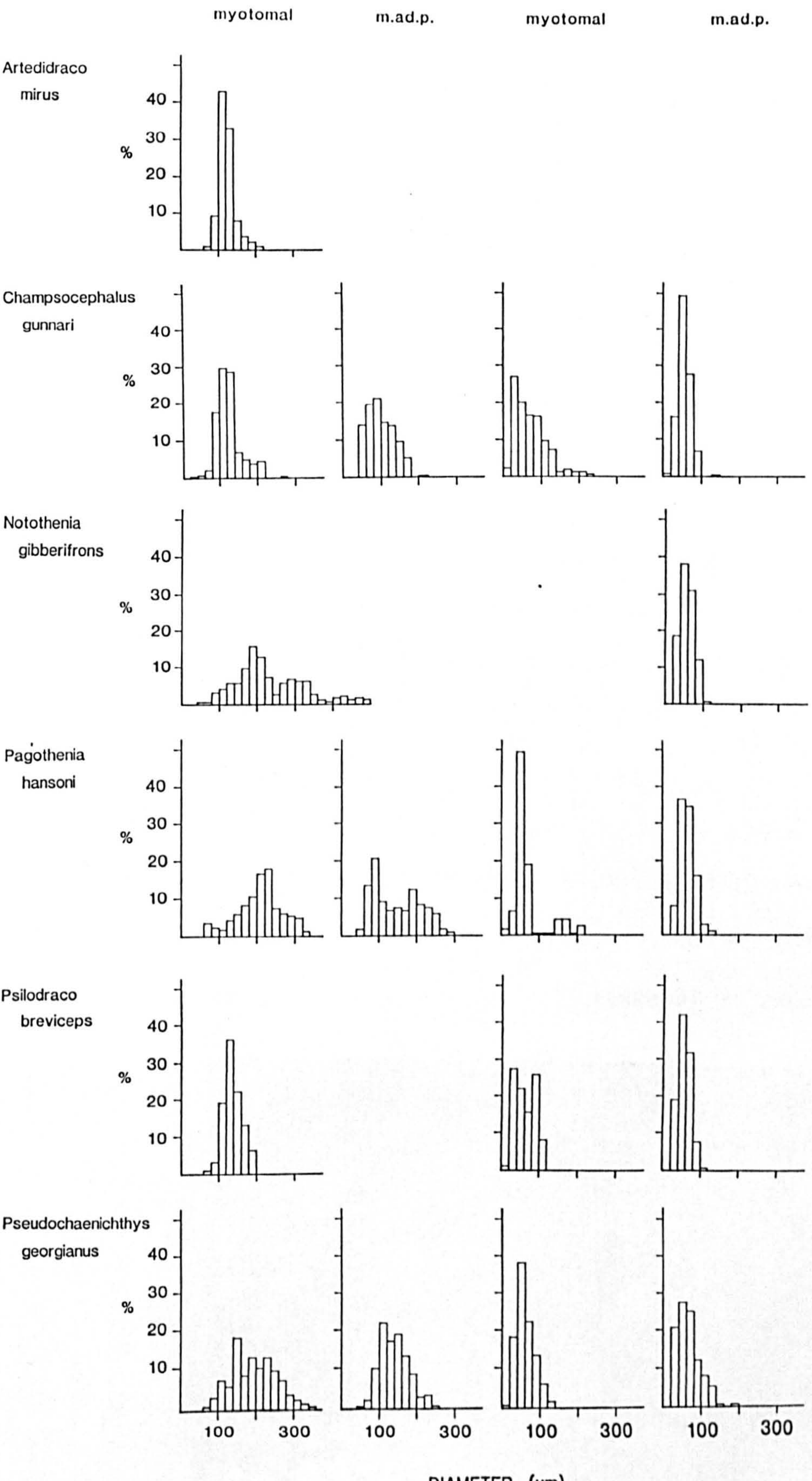

FAST MUSCLE FIBRES
SLOW MUSCLE FIBRES
myotomal
m.ad.p.
myotomal
m.ad.p.
Artedidraco mirus
Champsocephalus gunnari
Notothenia gibberifrons
Pagothenia hansoni
Psilodraco breviceps
Pseudochaenichthys georgianus
%
10
20
30
40
100
300
DIAMETER (µm)

Figure 2.5. *A. mirus*, post-larvae mid-trunk myotomal section, stained for α-GPDHase. F fast; S slow; T tonic? (Scale bar = 200μm).

Figure 2.6. *P. georgianus*, post-larvae myotomal section at region of lateral line, stained for α-GPDHase. F fast; S slow; T tonic? (Scale bar = 100μm).

Figure 2.7. *C. gunnari*, adult myotomal section, stained for SDHase. Illustrating the narrow band of slow fibres at the periphery of the trunk and the sharp distinction between slow (S) and fast (F) fibre types.
(Scale bar = 100μm).

FIG.2.5.

FIG.2.6.

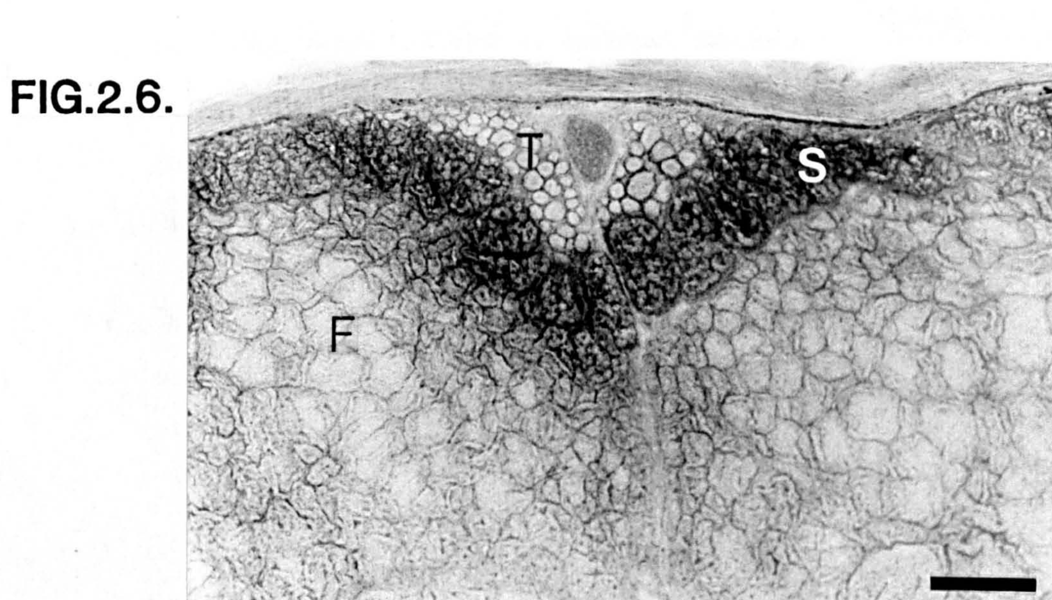


FIG.2.7.

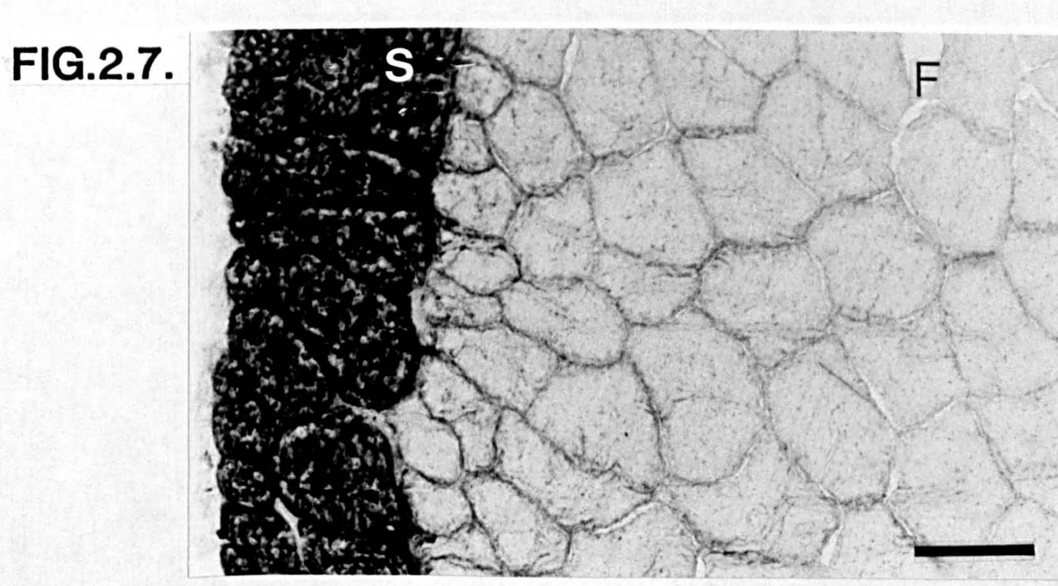


Figure 2.8. *P. breviceps*, adult myotomal section at level of the lateral line nerve, stained for SDHase. F fast; S slow (Scale bar = 100µm).

Figure 2.9. *P. breviceps*, adult myotomal section at the level of the lateral line nerve, stained for glycogen (PAS reagent). F fast; S slow (Scale bar = 200µm). Note the relatively large area of slow muscle fibres in the trunk of *P. breviceps*, illustrated in Figs. 2.8 & 2.9.

Figure 2.10. *P. georgianus*, adult myotomal section at the level of the lateral line nerve stained for SDHase. Note the large number of weakly staining tonic fibres in this region. S slow; T tonic?; N lateral line nerve (Scale bar = 200µm).

FIG.2.8.

FIG.2.9.

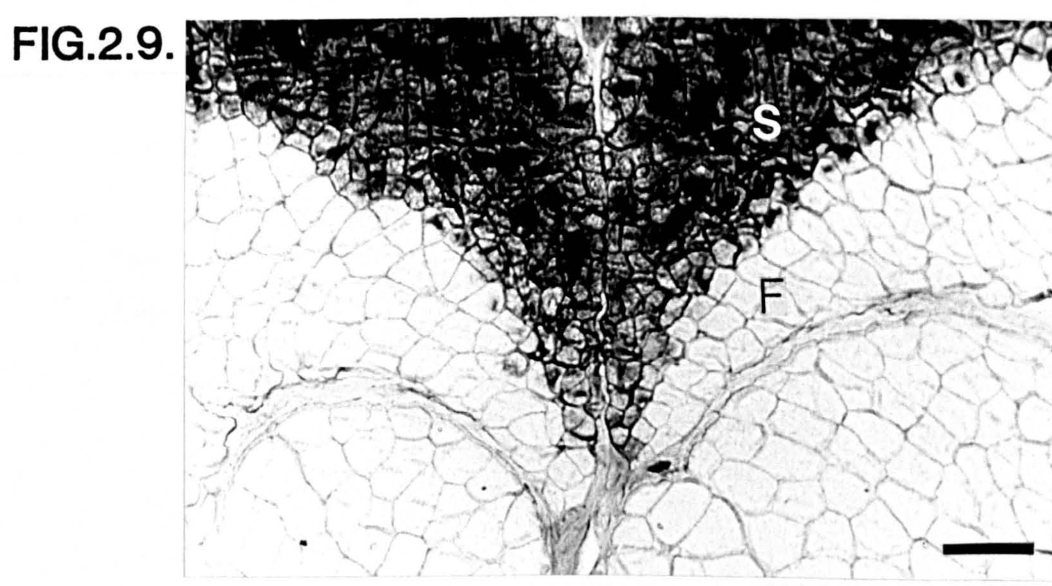


FIG.2.10.

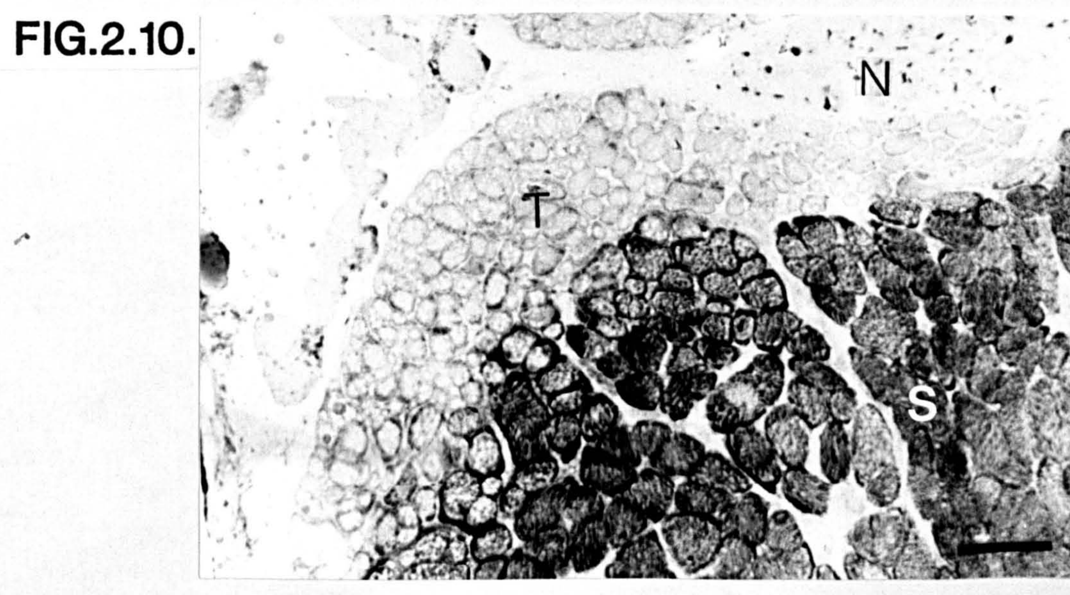


Figure 2.11. *C. gunnari*, adult m.ad.p. stained for SDHase. A narrow band of fast (F) fibres surrounds the periphery of the m.ad.pro. Darkly staining slow (S) fibres make up the larger proportion of the muscle (Scale bar = 200µm).

Figure 2.12. *P. hansoni*, adult m.ad.p. stained for lipid (Sudan Black B). Note the narrow band of fast (F) fibres on the periphery of the muscle and the relatively abrupt distinction between fibre types (Scale bar = 200µm).

Figure 2.13. *N. gibberifrons*, adult m.ad.pro. stained for SDHase. Note the mixture of muscle fibre types in this region of the m.ad.p. F fast; S slow (Scale bar = 100µm).

FIG.2.11.

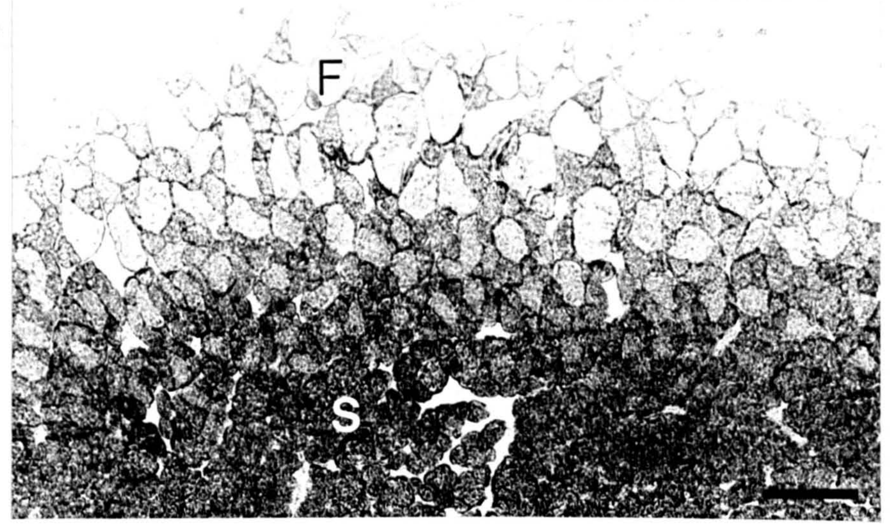


FIG.2.12.

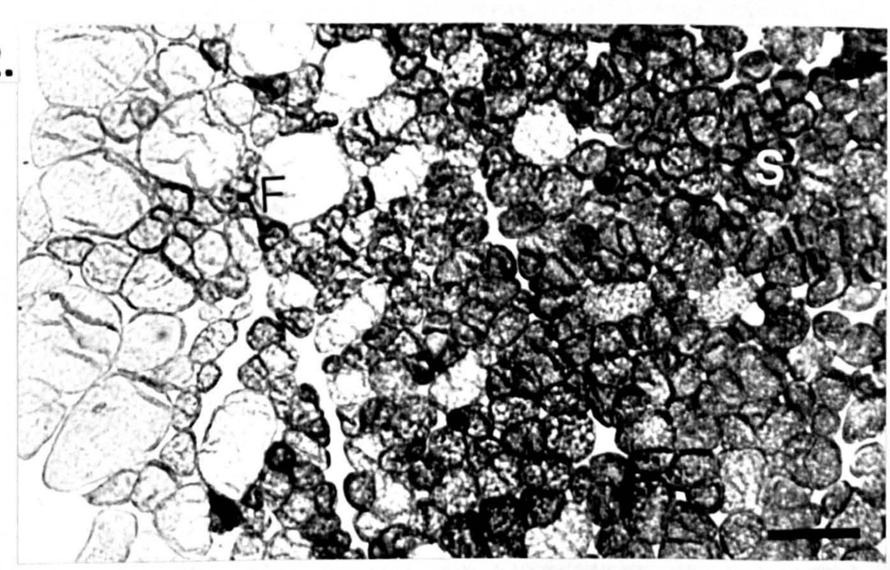
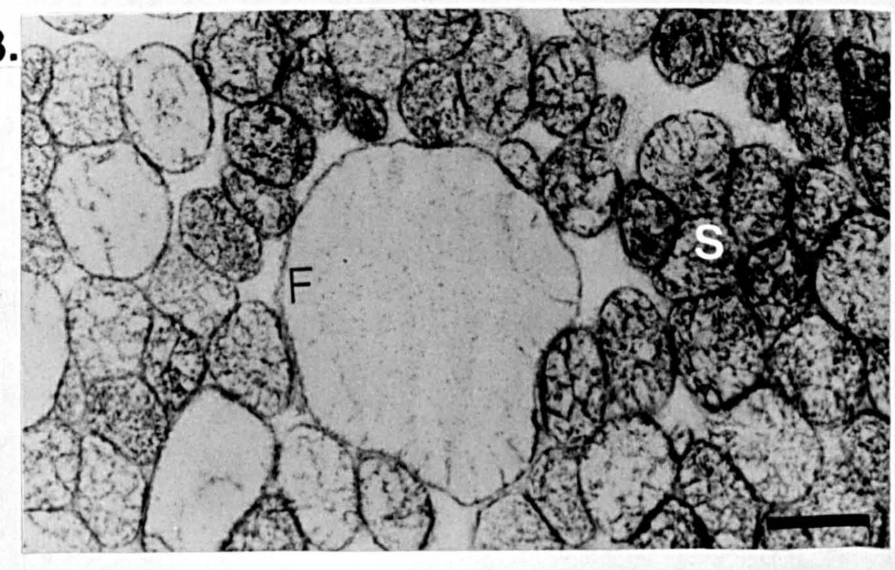


FIG.2.13.



CHAPTER 3.

KINEMATICS OF LABRIFORM AND SUBCARANGIFORM SWIMMING IN THE ANTARCTIC FISH, *NOTOTHENIA NEGLECTA*.

INTRODUCTION.

As discussed previously (Chapter 1), the *in vitro* contractile properties of muscle fibres vary in their dependence to temperature. Tension generation and "economy" of contraction are both partially compensated to low temperatures in notothenioids (for review see Johnston & Altringham, 1988a). However, not all contractile processes exhibit temperature compensation. For example, the unloaded contraction velocity of skinned muscle fibres shows little variation at 0°C between tropical, temperate and antarctic fish (Johnston & Brill, 1984).

There have been relatively few studies of temperature adaptation at higher levels of organisation (Macdonald *et al.* 1987). Two motor systems have previously been studied in relation to adaptation to freezing temperatures in notothenioids. Higher saccade velocity (rapid eye movement) reduces the period of visual disruption during eye movements (Montgomery & Macdonald, 1984). Similarly the capacity for maximum swimming speeds may determine both efficiency of prey capture and predator avoidance (Beamish, 1978). Strong selective pressures would be expected to maximise both saccade velocity and burst swimming performance.

In several antarctic species the velocity of the fastest eye movements at -1.9°C are about half that of temperate water fish acclimated to 14°C (Montgomery & Macdonald, 1984). Eye movements of warm temperate fish extrapolate to zero velocity at 3-4°C and cease at 5°C (Montgomery & Macdonald, 1983). The partial compensation in

for adaptation to low temperatures of the C.N.S. (see Macdonald *et al*. 1987). In contrast, maximum swimming performance of the cryopelagic species, *Pagothenia borchgrevinki* was similar to estimates from warm water fish extrapolated to 0°C (Montgomery & Macdonald, 1984). This limited swimming performance was attributed to a lack of capacity adaptation in the muscular system (Montgomery & Macdonald, 1984, McVean & Montgomery, 1987).

The aim of the present study was to obtain a detailed kinematic analysis of sustained and burst swimming behaviour in pelagic and demersal stages of the antarctic fish *Notothenia neglecta* Nybelin. The results are discussed in relation to similar data from temperate and tropical species.

MATERIALS AND METHODS.

Fish.

Notothenia neglecta (Nototheniidae; Notothenioidei; Perciformes) were obtained from the South Orkney Islands, Antarctica, and transported to St Andrews, Scotland. Fish were maintained in tanks of filtered recirculated sea water at +2°C within an air-temperature controlled cold-room. When not being used for swimming analysis, fish were fed twice a week to satiation on a diet of chopped squid, herring or krill.

Four adult fish (total length 26.9–30.5cm) and four juvenile fish (total length 7.1–8.1cm) were used.

Methods.

Fish were trained to swim along a 4m long raceway (Fig. 1), by association of an underwater flashing light with the appearance of food as described by Wardle & Kanwisher (1974). Three to four fish were kept in the raceway at a time to stimulate a competitive situation and induce burst swimming sequences. Spontaneous swimming along the raceway was also filmed and analysed.

A video camera (JVC KY-2700 CVC) was positioned 170cm above the base of the raceway to provide dorsal views of swimming sequences(Fig. 2). Lateral views were obtained by filming through a window in the side of the raceway. A background illumination lighting system (Wardle, 1975; Videler, 1981), together with reflex reflector material (Scotchlite 3M) on the base and side of the raceway produced dark silhouettes of the fish against a bright background (Fig. 2), while maintaining overall low light levels needed for the fish to perform.

Film analysis.

Two distinct swimming modes were examined, labriform and subcarangiform motion. The transition from labriform to subcarangiform swimming was also examined in the adult fish.

Video recordings (50 frames s^{-1}) were analysed frame by frame, with a dubbed time interval of 0.02 s. A 5cm grid was marked on the base and side of the raceway, the scale was accordingly corrected for the level at which the fish swam, and movement recorded with reference to the grid. Forward velocity was measured along an x-axis, the path of

motion; x values of the tip of the head were obtained for each frame of a swimming sequence. Tail-beat frequency, tail-beat amplitude and stride length (the distance moved over one complete tail-beat cycle) were measured for each sequence of steady subcarangiform swimming (Fig. 3A).

Kinematic parameters recorded during labriform motion include pectoral fin beat frequency, pectoral fin adduction time and the stride (the distance moved over one complete pectoral fin beat cycle). Measurements of adduction time of the leading edge of the pectoral fin and amplitude of the fin beat were also possible in the adult fish.

Statistical analysis.

Velocity in the x direction was estimated to be the mean value of five dx/dt values around t = n :

$$dx/dt_{=n} = f(1/12x_{(n-2)} - 2/3x_{(n-1)} + 2/3x_{(n+1)} - 1/12x_{(n+2)}),$$

where f is the number of frames per second (Videler & Wardle, 1978). Labriform swimming velocity was estimated at 0.04 s intervals (f = 25), analysis of subcarangiform motion was carried out at 0.02 s intervals (f = 50). Only steady swimming sequences with a standard error (S.E.) of mean forward velocity values of less than 5 percent were used for analysis of labriform and subcarangiform motion.

The five fastest subcarangiform swimming sequences and the five fastest sequences of labriform swimming were obtained for each fish. These provided mean fastest subcarangiform and labriform motion parameters for each fish (N = 5), and for each size group (N = 20).

Comparison of length-specific swimming velocity and kinematics was made between juvenile and adult fish. A nested two-way analysis of variance (Sokal & Rohlf, 1981), was carried out to determine the significance of variation between fish within each size and between size groups. Because of the low number of steady swimming sequences obtained for fish no. 6; in order to obtain a balanced design for the nested two-way analysis of variance, one set of data from the adult fish was dropped. This provided data from three fish of each size group, with one missing term for fish no. 8.

RESULTS.

Description of the labriform stroke.

Sustained low-speed swimming is achieved by using large, fan-shaped pectoral fins, in a drag-based, labriform mode of locomotion. The pectoral fin beat pattern is similar to pattern A described for *Cymatogaster aggregata* (Webb, 1973).

The main power stroke involved in labriform motion is the adduction phase (Fig. 4 A-D). Successive fin rays, joined by a highly flexible membrane, move both laterally backwards and vertically upwards, producing a sinusoidal wave over each pectoral fin. Once adducted, the leading anterior edge of the pectoral fin continues to move dorsally, against the side of the body, before the abduction stroke commences. Abduction of the fin involves both laterally forward and vertically downward motion of the fin rays. Abduction occurs in two stages. Initially the

fin is abducted to a glide position (Fig. 4E) and just prior to adduction, the leading edge moves anteriorly and ventrally again to begin the adduction stroke (Fig. 4A).

Unlike in *C. aggregata*, the main refractory period of the fin beat cycle in *N. neglecta* occurs when the fin is abducted, not adducted. This may in part be due to the negative buoyancy of *N. neglecta*, as the abducted pectoral fin provides lift during the glide period.

<u>Labriform swimming kinematics.</u>

The kinematics of the five fastest labriform sequences observed for each fish are summarized in Table 1. One of the fingerling fish was only filmed once during a steady labriform swimming sequence and is therefore not included in the table. Fingerling fish no. 8 was only filmed four times during steady labriform motion, so that the values listed for that fish are not necessarily the fastest speeds obtainable.

Forward swimming velocity was significantly different among individuals within each size group ($P \leq 0.05$). Despite this, a nested analysis of variance indicates that significant variation between size groups also occurred ($P \leq 0.01$). Juvenile fish accomplished length-specific speeds using labriform swimming (1.36 ± 0.26 Ls^{-1}) that were nearly twice those obtained for the adult fish (0.78 ± 0.12 Ls^{-1}) ($P \leq 0.01$). Pectoral fin beat frequencies and pectoral fin adduction times were also significantly shorter in juvenile than in adult fish ($P \leq 0.01$). However the length-specific distance moved forward during a fin beat cycle, the stride, was not significantly different between the size groups

(Table 1). The transparent nature of the juvenile pectoral fin made more detailed analysis of the pectoral fin beat cycle difficult with the filming technique used.

Subcarangiform motion.

Faster swimming speeds are achieved by employing a subcarangiform mode of locomotion. Pectoral and pelvic fins are adducted against the body, the dorsal and anal fins are part erected (Fig. 3).

A summary of the burst swimming kinematics of each of the fish studied is included in Table 2. No significant differences in kinematic parameter were found among fish of the same size group.

The fastest length-specific sprint swimming speeds were significantly higher in juvenile fish than in the adults ($P \leq 0.02$). The specific amplitude of the tail-beat was also significantly higher in the juvenile fish ($P \leq 0.02$). Higher tail-beat frequencies and strides were observed in the juvenile fish, although these were not significantly higher at the $P \leq 0.5$ level (Table 2).

Labriform to subcarangiform transition.

Several sequences of the transition from labriform to subcarangiform motion were analysed for adult *N. neglecta*. The response of fish provoked by flashing of the underwater light was analogous to fast starts described by Webb (1978) and Weihs (1973). A preparatory stroke involving movement of the trunk into a "C-start" position was coupled to rapid adduction of the pectoral fins. Caudal fin propulsive strokes of high amplitude accelerated the fish to a maximum

velocity. In the majority of cases, a maximum velocity was only reached after at least two complete tail-beat cycles (Fig. 5). During a typical fast start, maximum velocity is reached at the end of kinematic stage 2 (the first propulsive stroke), with maximum acceleration occurring during stage 1 (Webb, 1978).

Fastest acceleration was observed during a transition sequence performed by fish no. 4 (total length 30.2cm). Acceleration over two strides was 301 cms^{-2}, tail-beat frequency was 7.14 Hz and amplitude 0.21L. Full adduction time of the pectoral fins (0.22s) was considerably quicker than that observed during steady labriform motion.

Range of swimming mode.

A comparison of range of swimming speeds at which different steady swimming modes are employed in juvenile and adult fish is shown in (Fig. 6). The juvenile fish exhibit a greater range of steady subcarangiform speeds (2.3-8.4 Ls^{-1}), and tail-beat frequencies (4.0-12.5 Hz). Although no overlap between steady labriform and steady subcarangiform motion was observed, the gap between maximum pectoral and minimum caudal fin powered motion in the juvenile fish was small. In comparison, the adult fish have a distinct two-geared system, with steady pectoral powered motion reaching speeds of less than 1 Ls^{-1} and the lowest steady subcarangiform motion being 3.6 Ls^{-1}. Occasionally, adult fish used both swimming modes, consisting of several tail flicks of low amplitude, during normal labriform motion. This type of swimming behaviour was not observed in

the juvenile fish, although at low subcarangiform speeds the pectoral fins were abducted.

DISCUSSION.

Developmental changes.

Following an inshore migration the pelagic blue fingerling stage of *Notothenia neglecta* undergoes a gradual transition *via* brown fingerling and juvenile stages to the demersal adult form with associated changes in pigmentation and body morphology (Norman, 1938). Adults are ambush feeders and can be observed perching amongst rocks or partially burying themselves in soft mud in wait for a prey organism to approach (Daniels, 1982). In contrast, juvenile *N. neglecta* spend long periods in the water column. Juveniles are not recruited into the demersal population until they are between 1 and 2 years of age and over 10 cm in standard length (Everson, 1970). The transition from a pelagic fingerling to a demersal adult stage is also associated with a major structural remodelling of the skeletal muscle of the myotomes. For example, slow fibres constitute up to 24% of the cross-sectional area of myotomes in fingerlings compared with only 3% in adults (Johnston & Camm, 1987). The consequences of these changes in life-style and locomotory musculature on the swimming performance and behaviour of *N. neglecta* are discussed.

Locomotion of Adult fish.

Adult *N. neglecta* use the labriform mode of swimming almost exclusively at slow sustained speeds. Low-amplitude beats of the tail are occasionally used to increase the

forward velocity slightly, during labriform motion (Fig. 6). Harrison *et al.* (1987) have made a detailed study of the anatomy and histochemical characteristics of the pectoral fin muscles, which together comprise 2.5% of the total body weight. The main propulsive phase, the adduction stroke (Fig 4), is powered by the m. adductor profundis , the largest of the pectoral muscles. All six pectoral muscles play a part in articulating the complex dorsal and ventral movements of the fin during adduction and abduction, respectively (Harrison *et al.* 1987). It is clear that there must be a phase difference in activation and contraction of different parts of the m. adductor profundis of the same duration as that between the leading and trailing fin rays during a pectoral fin beat. The muscles of the pectoral fins in both adult and juvenile fish are composed mainly of slow fibres of small diameter (24.2-42.5μm) and relatively high capillary numerical (500-1730mm^{-2}) and mitochondrial volume (34-37%) densities (Johnston & Camm, 1987). The rapid adduction of the fins during the transition from labriform to subcarangiform motion involves a power stroke (Fig. 5) and may be associated with recruitment of the large-diameter, fast fibres found on the periphery of the adductor muscles (Harrison *et al.* 1987).

Fastest swimming speeds during labriform motion are not necessarily maximum sustainable speeds. Two species that use labriform motion at low speeds, *Cymatogaster aggregata* (Webb, 1973) and *Lepomis gibossus* (Brett & Sutherland, 1969), achieve higher critical swimming speeds by the use of subcarangiform motion. However, the

cryopelagic antarctic fish *Pagothenia borchgrevinki*, 23cm total length, have 15min critical swimming speeds (Forster *et al.* 1987) of the same value (1.8 Ls^{-1}) as the maximum pectoral fin powered speeds observed in static water (Montgomery & Macdonald, 1985). This suggests that there is only a limited contribution to aerobic powered swimming by the trunk muscles in *P. borchgrevinki*.

Some notothenioids have adopted a secondarily pelagic mode of life as adults (Andriashev, 1987). Studies of the swimming behaviour of *P. borchgrevinki* (Montgomery & Macdonald, 1985) and of muscle fibre distribution in *Pseudochaenicthyis georgianus* and *Chamsocephalus gunnari* (Dunn *et al.* 1988) suggest that these secondarily pelagic species also rely on labriform swimming at sustained speeds. It has been suggested that pectoral drag-based mechanisms of propulsion are an adaptation to slow swimming, where the efficiency of the subcarangiform mode is low (Blake, 1979).

The almost total reliance by adult *N. neglecta* upon labriform motion for sustained swimming observed in this study (Fig. 6) questions the role of the myotomal slow muscle. These slow muscle fibres are of larger diameter (92.0µm) and lower mitochondrial content (13.1%) than those found in the pectoral fin muscles (Johnston & Camm, 1987). Histochemical studies of other demersal nototheniods also suggest that the myotomal slow muscle has a lower aerobic capacity than the pectoral fin muscles (Davison & Macdonald, 1985; Dunn *et al.* 1988). Turning movements during labriform motion involve using the tail as a rudder and the slow muscle of the myotomes may play a part in

bending the trunk. The low-amplitude tail flicks occasionally observed during labriform motion may also involve recruitment of the myotomal slow muscle.

The very small proportion and relatively low aerobic capacity of slow muscle in the trunk of adult *N. neglecta* result in a swimming behaviour in which the trunk muscle is utilized almost solely for anaerobic power output. Anaerobic energy production is supplied largely *via* an enhanced creatine phosphate hydrolysis pathway, the glycolytic capacity of the trunk muscle of adult *N. neglecta* being relatively reduced (Dunn & Johnston, 1986). This metabolic organization is geared to rapid, short-term rates of ATP production, but limits the burst endurance capacity of adult *N. neglecta*.

Accelerations attained and the duration of the kinematic stages during transitions from labriform to subcarangiform motion in *N. neglecta* (Fig. 5) are not comparable to the "Mauthner-initiated" startle responses, which are involved in avoidance reactions (Eaton *et al.* 1977; Webb, 1976). The acceleration produced by the large-amplitude, rapid tail-beats during fast-starts, is obviously important to prey capture during ambush feeding of adult *N. neglecta*; these feeding bursts are probably of short duration. Constraints on burst endurance imposed by utilizing the phosphocreatine pathway of anaerobic energy supply would be expected to affect predator avoidance to a greater extent than prey capture in adult *N. neglecta*.

Locomotion of juvenile fish.

The cross-sectional area of slow muscle in the trunk of juveniles *N. neglecta* is of similar proportion (Johnston & Camm, 1987) to that found in small pelagic species from warmer waters (Greer-Walker & Pull, 1975). In contrast, the myotomes of adult stages contain very little slow muscle (≥3%) (Johnston & Camm, 1987). The higher proportion of slow myotomal muscle in juvenile *N. neglecta* allows them to use subcarangiform swimming at speeds around $2Ls^{-1}$ (Fig. 4), much slower than observed in adults. Slow fibres of the myotomes in juveniles have a similar fine structure to those of the pectoral fin muscles with high capillary numerical densities ($1225mm^{-2}$) and high volume densities of mitochondria (37.0 %) (Johnston & Camm, 1987).

Smaller fish are capable of higher length-specific swimming speeds due, in part, to faster tail-beat frequencies (Bainbridge, 1958; Hunter & Zweifel, 1971). Size-related variations in body wavelength, tail-beat amplitude and depth of the trailing edge of the tail also determine the thrust produced by fish of different sizes (Webb *et al.* 1984). Highest tail-beat frequencies of juvenile *N. neglecta* appear to be particularly slow; the variation between adult and juveniles is not significant at the 0.05 probability level (Table 2), despite significantly different fastest swimming speeds (body lengths per second) ($P \leq 0.02$).

Temperature adaptation of swimming performance.

It has been suggested that isotonic twitch contraction times can be used to predict maximum swimming speeds

(Wardle, 1975). Twitch contraction times of myotomal muscle of *P. borchgrevinki* at -1.9° C, are only slightly shorter than for various temperate species extrapolated to the same low temperature (McVean & Montgomery, 1987). This has led some authors to predict that the burst swimming speeds of antarctic fish do not show capacity adaptations to low temperature (Macdonald *et al.* 1987). However, such analyses do not take into account many dynamic properties of muscle relevant to locomotion: for example, the number of cycles of stimulation and the effects of stretch on subsequent contractions (Altringham & Johnston, 1988a). Although tail-beat frequency may place an upper boundary on the maximum swimming speed, it is only one of a number of parameters involved; others include muscle force, power and endurance (Johnsrude & Webb, 1985; Webb & Johnsrude, 1988). It is clear that some of these parameters exhibit more substantial adaptations to low temperature. For example, studies with demembranated fibres have shown that the power output of fast muscle is 6-10 times higher for antarctic than for tropical species at 0° C (Johnston & Altringham, 1985). This largely reflects the higher maximum tensions that fibres from antarctic fish generate at low temperatures (Johnston & Brill, 1984; Johnston & Altringham, 1985).

Comparisons of the maximum observed burst swimming speeds of antarctic, temperate and tropical fish are complicated by the variety of methods of analysis used and the limited data available. Values shown in Table 3 were measured over a similar duration to those in this study, in essentially static water, and they are from fish of

comparable size. However, none of the data is directly comparable to that of *N. neglecta*. Values of maximum swimming speeds of tropical species are limited to measurements on large pelagic scombrid species (Walters & Fierstein, 1962; Yuen, 1966). Body form is an important determinant of maximum swimming speed, the lateral profile (Fig. 3) of *N. neglecta* is of a design maximizing thrust, with large dorsal and anal fins that give the fish a deep silhouette along the length of the body (Webb, 1984). Typical of many demersal species *N. neglecta* has a large head and relatively small trunk and myotomal muscle constitutes only 30% of the total body weight (Harrison *et al.* 1987). In comparison the myotomal muscle of trout, *Salmo gairdneri*, 30cm in total length, makes up 58% of the total body weight (Webb, 1976).

In spite of the limitations of the data on the maximum swimming speeds of fish of similar body shape and behaviour, it is clear that there is some overlap between antarctic and temperate fish more than 20cm long. It seems likely that adult *N. neglecta* are able to swim as fast as similar bottom-living fish from temperate environments. However, the higher length-specific swimming speeds and tail-beat frequencies observed in smaller temperate fish (Table 3) are not matched by juvenile *N. neglecta*. The relatively low tail-beat frequencies in juvenile *N. neglecta* may in part be compensated for by significantly greater specific tail-beat amplitudes (Table 2). However, it seems likely that the poor burst swimming performance of juveniles reflects incomplete adaptation of the myotomal muscle to low temperature.

TABLE 3.1. Kinematics of labriform swimming in the Antarctic teleost *Notothenia neglecta*. Values represent Mean ± S.E. Measurements are of the five fastest labriform sequences from each of 4 adult and 3 juvenile fish.

Fish No. (L.cm)	Forward velocity (L/s)	Forward velocity (cm/s)	Pectoral fin adduction time (s)	Leading edge adduction time (s)	Pectoral fin beat frequency (Hz)	Amplitude (rad)	Stride length (L)
Adult fish.							
1 (30.5)	0.70±0.07	21±2	0.35±0.02	0.23±0.02	1.02±0.18	1.79±0.10	0.68±0.09
2 (29.5)	0.72±0.09	21±3	0.35±0.04	0.21±0.04	1.03±0.11	1.83±0.06	0.70±0.11
3 (26.9)	0.85±0.11	23±3	0.34±0.04	0.19±0.03	1.02±0.11	1.86±0.08	0.80±0.02
4 (30.2)	0.86±0.09	26±3	0.31±0.02	0.20±0.03	0.95±0.09	1.82±0.21	0.91±0.07
Mean. (L) 29.3±1.6	0.78±0.12	23±0.3	0.34±0.04	0.21±0.03	1.01±0.13	1.83±0.12	0.77±0.12
Juvenile fish.							
5 (8.1)	1.59±0.12	13±1	0.21±0.02		1.72±0.25		0.93±0.13
7 (7.2)	1.23±0.22	9±2	0.23±0.05		1.70±0.29		0.73±0.18
8*(7.1)	1.24±0.26	9±1.8	0.20±0.02		1.70±0.30		0.69±0.16
Mean. (L) 7.5±0.6	1.36±0.26	10±2	0.21±0.03		1.71±0.26		0.78±0.18

* n = 4.

TABLE 3.2. Kinematics of burst swimming in the Antarctic teleost *Notothenia neglecta*. Values represent Mean ± S.E. Five sets of measurements were made from each of 4 fish of each size range.

Fish No.(L.cm)	Forward velocity (L/s)	Forward velocity (cm/s)	Tail-beat frequency (Hz)	Tail-beat amplitude (L)	Stride length (L)
Adult fish.					
1(30.5)	4.2±0.2	128±5.8	6.3±0.2	0.20±0.02	0.67±0.05
2(29.5)	4.2±0.3	127±9.8	6.1±0.8	0.19±0.04	0.70±0.04
3(26.9)	4.7±0.5	127±12.5	7.1±0.9	0.21±0.03	0.67±0.06
4(30.2)	4.1±0.4	123±11.6	6.9±0.2	0.18±0.02	0.59±0.06
Mean (L) 29.3±1.6	4.3±0.4	126±9.7	6.6±0.6	0.20±0.03	0.66±0.06
Juvenile fish.					
5(8.1)	6.2±1.0	50±7.8	10.0±1.5	0.27±0.01	0.63±0.13
6(7.9)	6.7±1.0	53±8.2	8.3±0.9	0.29±0.03	0.81±0.12
7(7.2)	6.8±0.4	49±3.3	8.2±1.2	0.29±0.04	0.83±0.07
8(7.1)	7.4±1.3	53±9.7	9.1±1.5	0.31±0.05	0.82±0.12
Mean (L) 7.6±0.5	6.8±1.0	51±7.2	8.9±1.4	0.29±0.04	0.77±0.14

TABLE 3.3. Comparitive data on the maximum swimming speeds and tail beat frequencies of Antarctic and temperate water fish.

(A) Adult fish.

Species	Total length (cm)	Maximum velocity (L/s)	Tail-beat frequency (Hz)	Environmental temperature. (° C)	Source
Notothenia neglecta	26.9	5.4	8.3	antarctic (2° C)	this study
Pagothenia borchgrevinki	23	4.9	5.7	antarctic (-1.8° C)	(1)
Leucicus leucicus (Dace)	24	9.2	13	temperate	(2)
Salmo gairdneri (Trout)	29.3	10.8	17	"	(2)
Carassius auratus (Goldfish)	22.5	3.6	5	"	(2)
Salmo salar (Salmon)	25-28	10	16.7	temperate 12° C	(3)
Pollachius virens (Saithe)	40	6.2	6.7	temperate	(4)
Scomber scombrus (Mackerel)	34	18	18	temperate 12-14° C	(5)
Sebastes mystinus (Blue rockfish)	15.1	7.0	N.D.	warm temperate	(6)

(B) Juvenile fish.

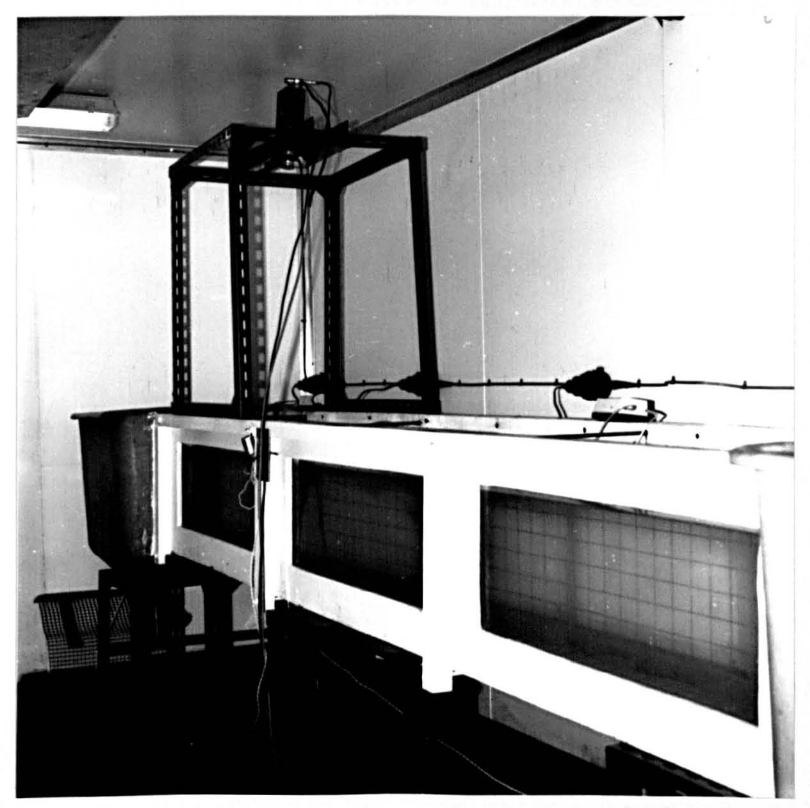
Species	Total length (cm)	Maximum velocity (L/s)	Tail-beat frequency (Hz)	Environmental temperature. (° C)	Source
Notothenia neglecta	7.2	8.7	11.1	antarctic (2° C)	this stud
Leucisus leucisus	9.0 (Dace)	17.8	25	temperate	(2)
Carassius auratus (Goldfish)	7.0	9.5	16	"	(2)
Melanogrammus aegelfinus (Haddock)	10	26	25	temperate (12° C)	(3)
Cymatogaster aggregata (Surf perch)	9.3	10.9	N.D.	warm temperate (15–20° C)	(6)
Chromis punctipinus (Blackfish)	8.5	11.1	N.D.	"	(6)
Trachurus symmetricus (Jack mackerel)	6.4	21.7	17	warm temperate (17–19° C)	(7)

References

(1) Montgomery & Macdonald, (1984).
(2) Bainbridge, (1958).
(3) Wardle, (1975).
(4) Videler & Hess, (1984).
(5) Wardle & He, (1986).
(6) Dorn et. al, (1979).
(7) Hunter & Zweifel, (1971).

Figure 3.1.

Vertical and horizontal views of the 4m long raceway along which fish were trained to swim by association of an underwater flashing light with the appearance of food. A video camera is positioned above the raceway to provide dorsal views of the swimming fish.



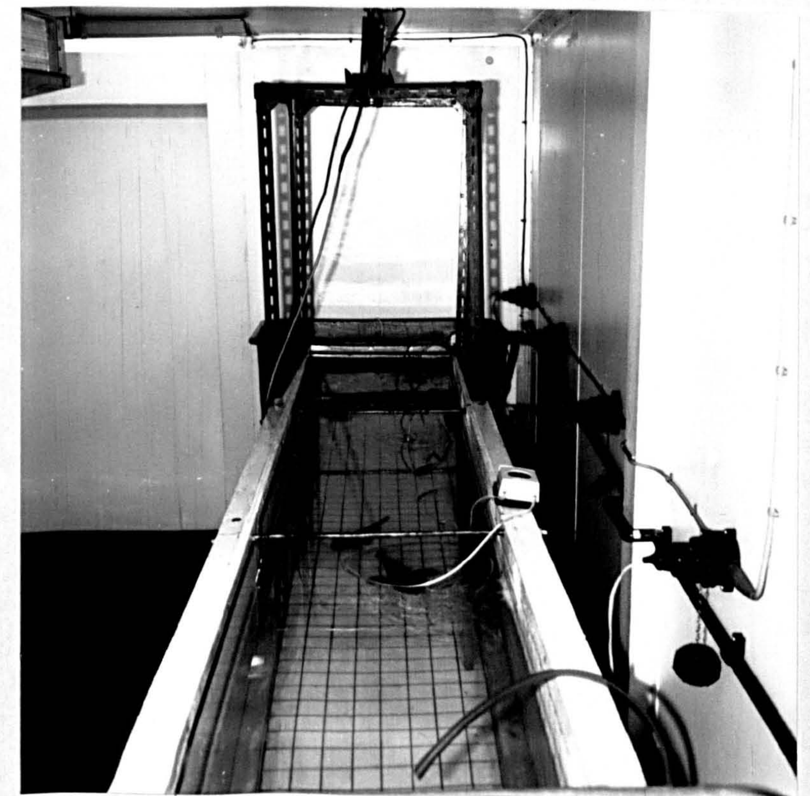
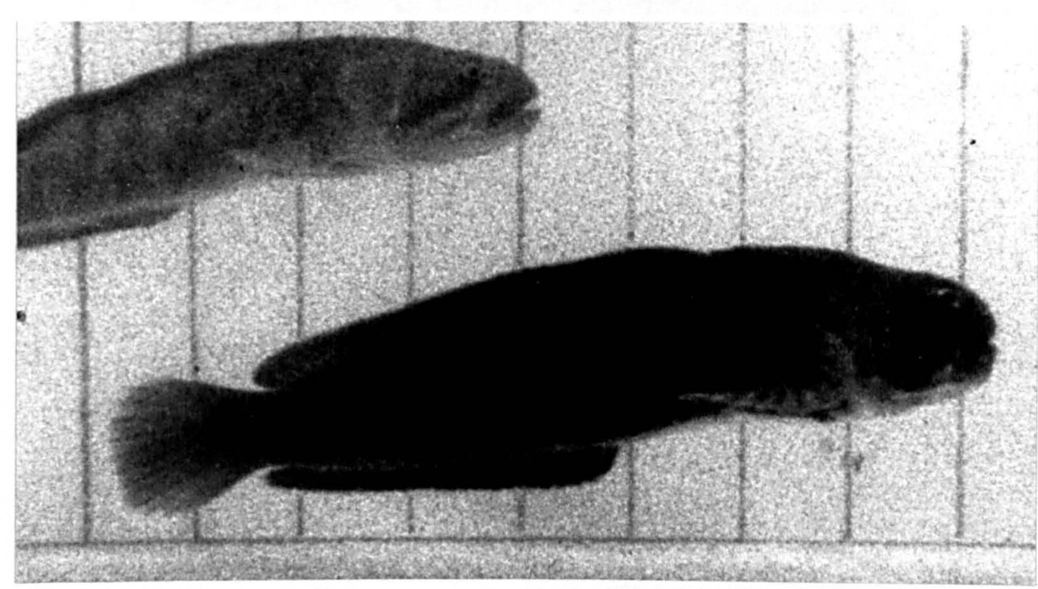


Figure 3.2.

Photographs illustrating the dark silhouettes of fish produced from a background illumination lighting system and reflex reflector material. (A) lateral view of *N. neglecta* during spontaneous labriform motion. (B) dorsal view of *N. neglecta* swimming in subcarangiform mode, towards a flashing light.

(A)



(B)

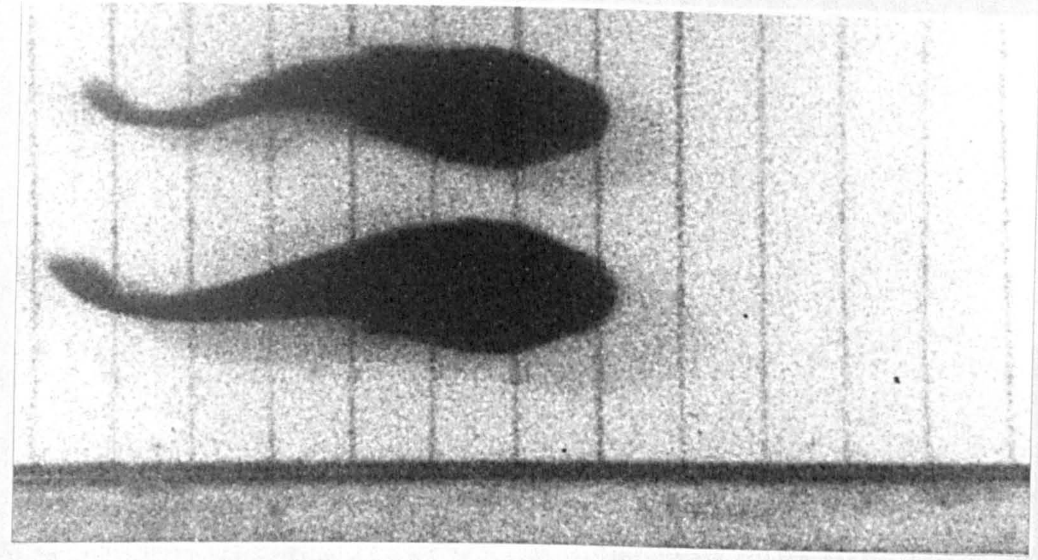


Figure 3.3.

Diagrams illustrating subcarangiform motion in *N. neglecta*. (A) Adult: lateral and dorsal profiles during burst swimming. Tracings of dorsal profiles were made at positions of maximum amplitude of the tail during a two stride sequence. Fish total length 30.5 cm, forward velocity 4.5 Ls^{-1} (136 cms^{-1}), tail-beat frequency 6.4 Hz, amplitude 0.19 L and stride 0.70 L.
(B) Juvenile: lateral body profile when stationary, dorsal profile of body positions at 0.02 s intervals during a complete tail-beat cycle or stride. Fish total length 7.1 cm, forward velocity 7.0 Ls^{-1} (50 cms^{-1}), tail-beat frequency 8.3 Hz, amplitude 0.27 L and stride 0.85 L.

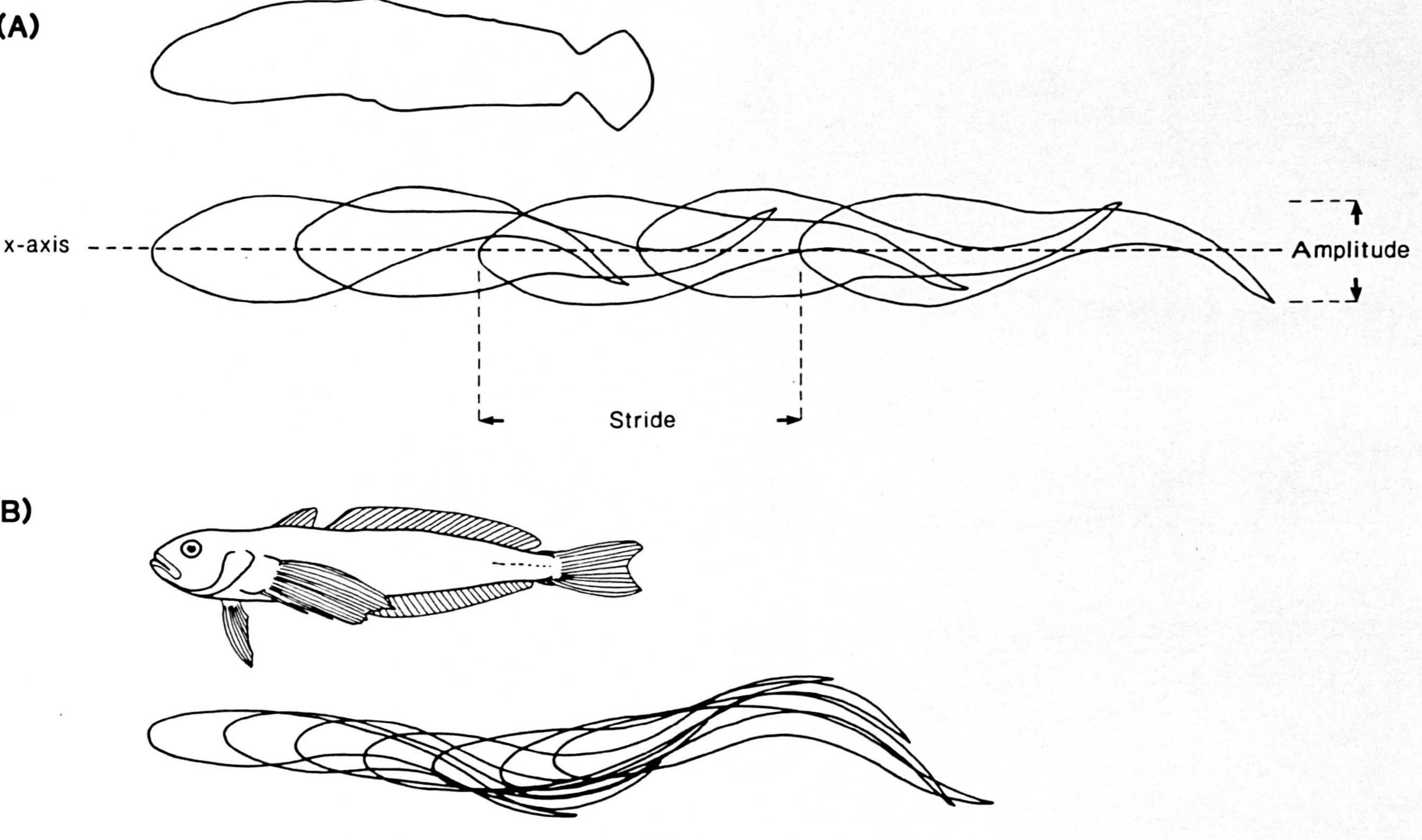

(A)
x-axis
Amplitude
Stride
(B)

Figure 3.4.

Diagram illustrating labriform motion in adult *N. neglecta*. The forward velocity over two complete pectoral fin-beat cycles, and a diagrammatic representation of the pectoral fin-beat pattern are shown. A-C adduction time of the leading edge of the pectoral fin, A-D the complete adduction stroke, D-E abduction and E-A the glide period.

Forward velocity (cm/s).

30
25
20
15
10

A C D E A C D E

0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2 2.2 2.4

Time (s).

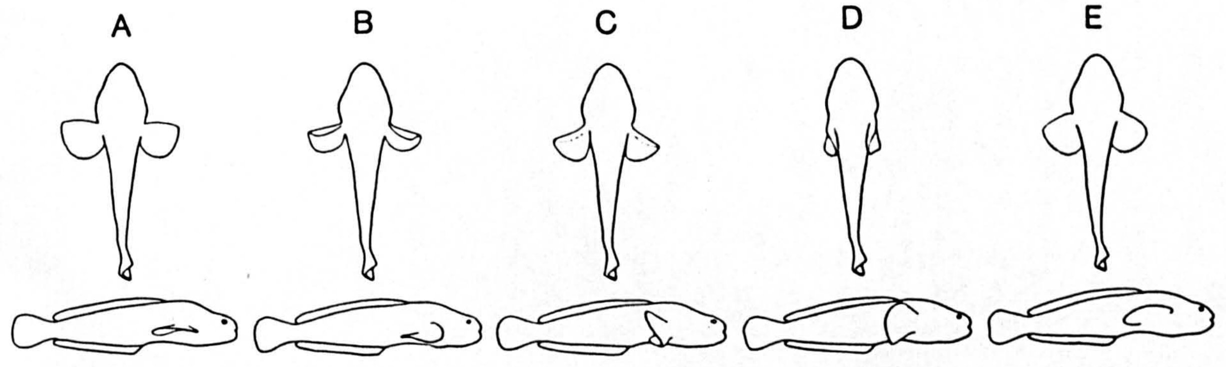


Figure 3.5.

Diagram of the transition from labriform to subcarangiform motion at the start of a burst swimming sequence, in an adult *N. neglecta*. Acceleration was calculated over the first two strides, first stride B–D (0.31 L), second D–E (0.51 L). Tail-beat frequency 5.9 Hz, amplitude 0.24 L. Pectoral fin adduction time (A–C) 0.24 s.

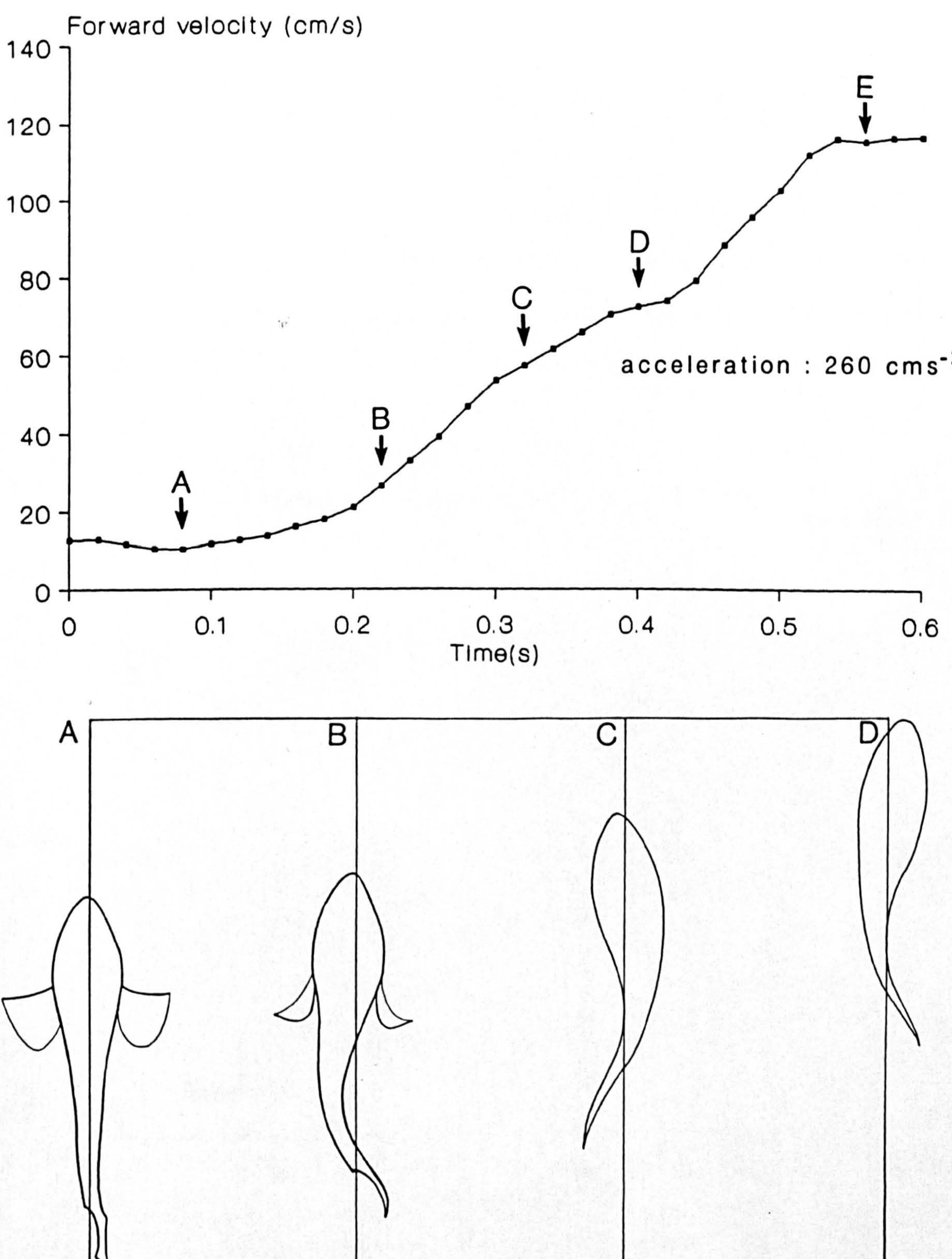
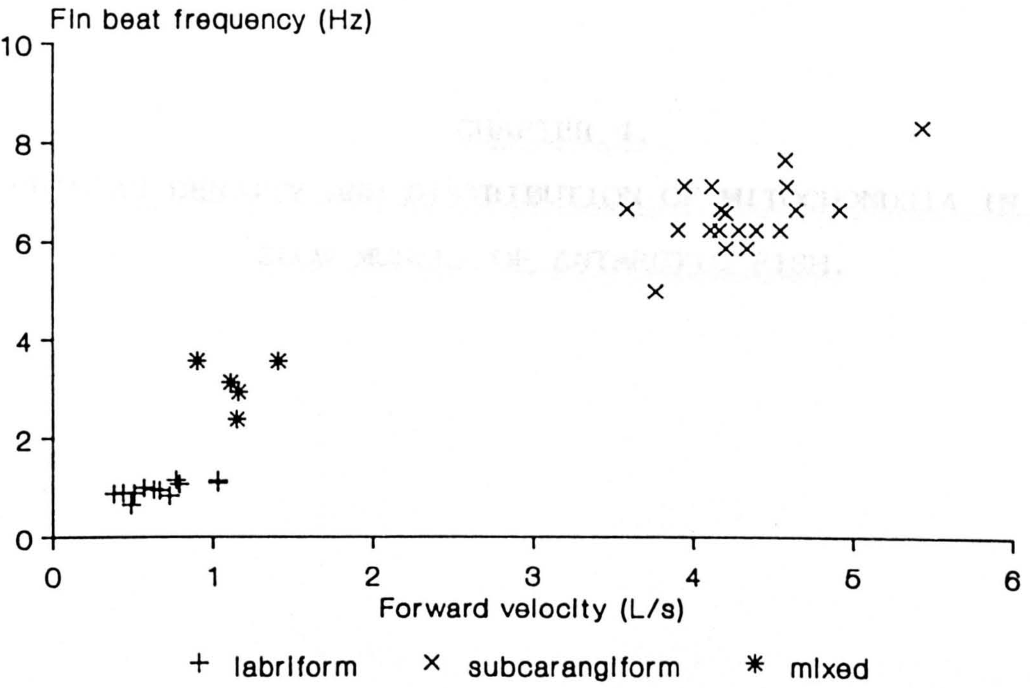
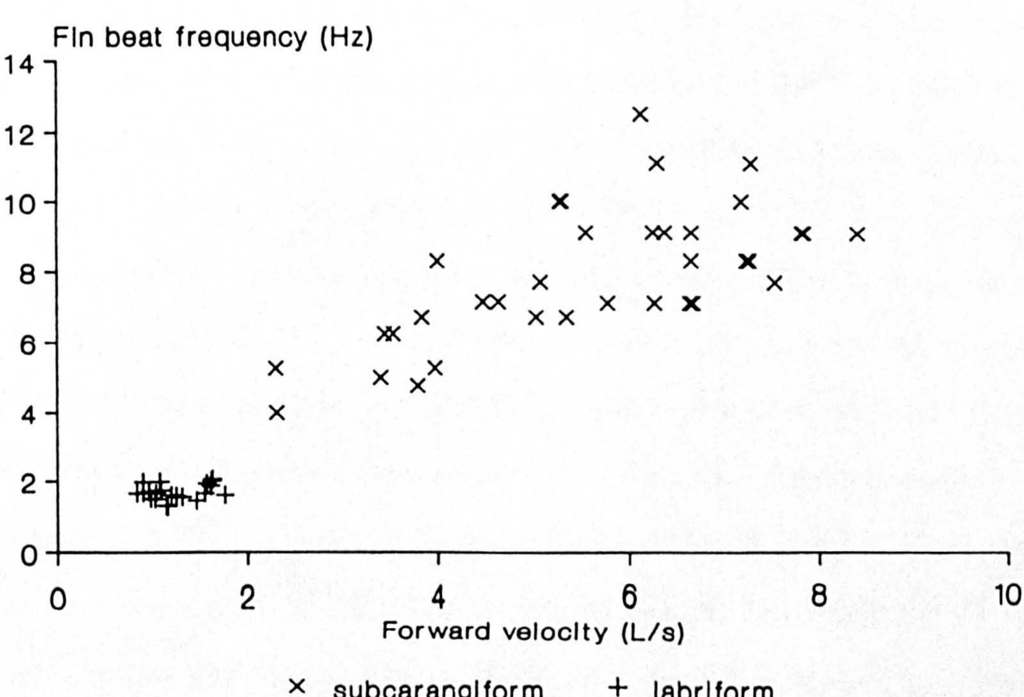

Forward velocity (cm/s)
140
120
100
80
60
40
20
0
0
0.1
0.2
0.3
0.4
0.5
0.6
Time(s)
A
B
C
D
E
acceleration : 260 cms^{-2}
A
B
C
D

Figure 3.6.

Swimming behaviour in adult (A) and juvenile (B) *N. neglecta*, illustrating the length-specific speeds at which different swimming modes are utilised. Points are single steady swimming sequences.



(B)



CHAPTER 4.

CRISTAE DENSITY AND DISTRIBUTION OF MITOCHONDRIA IN THE SLOW MUSCLE OF ANTARCTIC FISH.

INTRODUCTION.

Amongst mammals, skeletal muscle mitochondria would appear to have similar structural and functional characteristics. Even amongst species with a size related six-fold difference in aerobic capacity, the mitochondrial oxygen consumption falls between 3-5 ml O_2/min/ml mitochondria (Hoppeler & Lindstedt, 1985; Hoppeler *et al.* 1987). This allows mitochondrial volume density to be used as a descriptor of the oxidative capacity of the skeletal muscle fibres.

Two facts suggest that this is not the case in fish. Firstly, many antarctic fish are relatively sluggish, demersal species with relatively low active metabolic rates. Even the cryopelagic *Pagothenia borchgrevinki*, a comparatively active notothenioid, has a low aerobic scope for activity (Forster *et al.* 1987). However, slow muscle fibres in antarctic fish have high mitochondrial volume densities (V_v(mt,f) = 0.30-0.60), in comparison to fish from warmer waters (Walesby & Johnston, 1980; Fitch *et al.* 1984; Johnston & Harrison, 1987; Johnston & Camm, 1987; Johnston, 1987; Johnston *et al.* 1988).

Secondly, particularly high mitochondrial volume densities (V_v(mt,f) = 0.50) have been recorded in the slow muscle fibres of the haemoglobinless/myoglobinless *Chaenocephalus aceratus* (Johnston, 1987; Johnston & Harrison, 1987). However, the high mitochondrial volume density recorded in *C. aceratus*, is not matched by correspondingly high estimates of aerobic capacity. For example, the aerobic enzyme activities (Johnston &

Harrison, 1985) and oxygen consumption rates (Johnston, 1987) of slow fibres from the pectoral muscle of *C. aceratus* are slightly lower than in red-blooded notothenioids of similar activity. This suggests that oxygen consumption is lower per unit volume mitochondria in *C. aceratus* than in red-blooded species.

Many of the enzymes of the respiratory chain are embedded in the inner mitochondrial membrane/cristae. This study examines whether interspecific variations in oxygen consumption per unit mitochondria, are associated with differences in cristae density within the mitochondria.

Elevated mitochondrial content may reflect an increase in aerobic enzyme concentrations serving to partially compensate for catalytic limitations at cold temperatures (Sidell, 1980; 1983). High mitochondrial volume densities in cold-acclimated fish have also been associated with limitations to diffusion at low temperatures (Tytler & Sidell, 1985; Eggington & Sidell, 1986; 1989). In addition to mitochondrial content, diffusion of substrates to and from mitochondria depends upon mitochondrial distribution within muscle fibres and relative to capillaries (Mainwood & Rakusan, 1982; Kayar *et al.* 1986a; Kayar *et al.* 1986b; Kayar *et al.* 1988). In mammalian skeletal muscle fibres mitochondrial distribution shows a consistent pattern: a steep decline in mitochondrial volume density from capillaries towards the fibre centre and a shallow decline between capillaries at the fibre border.

The second major aim of this study was to examine the distribution pattern of mitochondria within the muscle

fibres of fish. Of particular interest is the relationship between mitochondrial distribution and possible diffusion limitations imposed by both low environmental temperatures and the lack of haemoglobin/ myoglobin.

Previous studies have demonstrated marked differences in the histochemical characteristics and distribution of muscle between notothenioid species with differing lifestyles and activities (Davison & Macdonald, 1985; Chapter 2). This study examined both demersal and pelagic species of antarctic fish, allowing the effects of differing activity pattern on muscle composition to be taken into account.

MATERIALS AND METHODS

Fish.

Four individuals from each of five species of Antarctic fish were used in this study. *Chaenocephalus aceratus* Lönnberg and *Notothenia gibberifrons* Lönnberg were obtained from the South Orkney Islands (60° 43'S,45° 36'W) during austral summer 1985/86. Fish were caught at a depth of 140-200m. *Champsocephalus gunnari* Lönnberg, *Psilodraco breviceps* Norman and *Protomyctophum bolini* Fraser-Brunner, were captured during the Offshore Biological Programme (7), austral summer 1986/87; carried out in the waters around South Georgia (54° S,36° 15'W). *C. gunnari* and *P. breviceps* were caught in near-shore waters at a depth of 0-200m. Specimens of *P. bolini* were caught at a depth of 1000-1200m.

Dragonets, *Callionymus lyra* L. captured in the Firth of Clyde, were provided by the Marine Biological Station, Millport.

The standard lengths, ecology and the normal environmental temperature of fish sampled, are given in Table 1.

Tissue sampling and preparation.

All the notothenioid species studied and *C. lyra* employ labriform motion at sustainable swimming speeds (Dunn *et al.* 1988; personal observations). Therefore, slow muscle fibres used for sustained swimming movements were dissected from the pectoral m. adductor profundis in these fish. Homologous fibres were obtained from the myotomal muscle bordering the lateral line nerve in *P. bolini*.

Small bundles of fibres were pinned via their insertions to cork strips and immersion fixed in 3% glutaraldehyde, 0.15mM phosphate buffer, pH 7.4 at 4° C. Samples were subsequently post-fixed in phosphate buffered 1% osmium tetroxide, rinsed, dehydrated in acetone and embedded in Araldite CY212 resin (EMscope, Ashford, England).

For each species, 5-10 blocks were prepared per fish and 4 blocks selected at random for sectioning.

Stereology.

Interspecific variation in muscle composition was analysed in four stages:

(1) Capillaries and fibres.

All sections were cut transverse to the longitudinal axis of the muscle fibres. Semi-thin (1.0-0.5um) sections, stained with toluidene blue, were used to determine fibre cross-sectional area a(f), fibre diameter d(f), capillary to fibre ratio C:F and capillary density $N_A(c,f)$. Measurements were made using the drawing arm of a microscope (Labophoto, Nikon, Japan) and a digital plannimeter interfaced to a microcomputer (Hewlett Packard 86B).

(2) Capillary dimensions and muscle fibre composition.

Ultrathin (approximately 80nm) sections were cut on a Reichert OM U2 ultramicrotome and mounted on 100 mesh pyroxyline coated copper grids. Sections were double stained with 2% aqueous uranyl acetate and Reynolds lead citrate. Micrographs were taken on a Philips 301 transmission electron microscope at 60kV.

The length density ($J_V(c,f)$), volume density ($V_V(c,f)$) and surface density ($S_V(c,f)$) of capillaries can be calculated from the capillary density ($N_A(c,f)$) provided the degree of anisotrophy (y^{-1}) is known:

$$J_V(c,f) = y^{-1} \cdot N_A(c,f)$$

$$V_V(c,f) = a(c) \cdot J_V(c,f)$$

$$S_V(c,f) = b(c) \cdot J_V(c,f)$$

The degree of anisotrophy was taken to be 1.02 for all species studied, based upon determinations made on *C.*

aceratus and *Notothenia neglecta* (Fitch *et al.* 1984). Micrographs of whole muscle fibres were taken at *750 and projected onto a counting grid at a final magnification of *2325. Volume densities (V_v) of myofibrils (mf), subsarcolemmal mitochondria (ms) and intermyofibrillar mitochondria (mi) were estimated using a point-counting method (Weibel, 1979). The intermyofibrillar zone was taken to include everything within the envelope drawn around the peripheral myofibril edges. A quadratic test system with 100-400 points per fibre and a lattice spacing of 4.3-2.6um was employed, such that grid spacing was approximately >1 and <1.5 times the average dimensions of myofibril and mitochondrial clusters.

(3) Intermyofibril zone composition.

Micrographs of the core of muscle fibres were taken at *4300. The intermyofibril mitochondrial and myofibril volume densities were estimated by point counting, using a template of 100 points covering an area of muscle fibre of $50um^2$, with a lattice spacing of 0.75um.. Surface densities of the outer mitochondrial membrane (S_v(mi)) were estimated from the same micrographs using a line-intercept system (Weibel, 1979). From these measurements mitochondrial spacing or mean free spacing (Λ_a), within the intermyofibril zone was calculated:

$$\Lambda_a = \frac{4(1-V_v)}{S_v}$$ (Weibel, 1980; Tyler & Sidell, 1984).

Micrographs of intermyofibril mitochondria chosen at random were taken at *43,000, cristae surface density (S_v(cr,mi)

was determined using a line intercept system with a grid spacing of 0.075um and 25-100 intersections per mitochondria. No corrections were made for the effects of section thickness or compression.

(4) Analysis of the distribution of mitochondria within muscle fibres.

In order to determine the distribution of mitochondria within the slow muscle fibres, specific zones were defined and analysed as described by Kayar *et al.* (1986).

Accurate positioning of the zones was made possible by obtaining micrographs of the whole cross section of muscle fibres at a magnification of *750. The necessary number of micrographs needed to cover a transect from the fibre border adjacent to a capillary to the fibre centre, were then taken at a magnification of *4300 (Fig. 1). In all three species studied, zone I covers an area extending from 0.0-6.75um from the fibre border and adjacent to a capillary. Zone II was defined as the intermyofibrillar area within zone I; the intermyofibrillar area being that within the envelope of the peripheral myofibril edges. In *N. gibberifrons* and *C. aceratus* zone III extended from 7.5-15.0um and zone IV between 15.0-36.0um, from the fibre border. The exact position of zone IV was dependant upon the fibre diameter, an attempt was made to obtain as central a position as possible. The smaller diameter of fibres in *C. lyra* required that to prevent overlap only three zones were defined. Zone III covered an area within the range 7.0-20.0um, again dependant upon fibre size.

Micrographs of the specific locations were projected on a counting grid at a final magnification of *13330. Volume and surface densities were measured using a square 100-point grid template, covering an area of 56um², with a lattice spacing of 0.75um. The parameters calculated in each zone included:

1. Total mitochondrial volume density (V_v(mt.z)).

2. Total myofibril volume density (V_v(mf.z)).

3. The combined volume densities of the other cell organelles including: nucleus, sarcoplasmic reticulum, lipid and sarcolemma (V_v(other.z)).

In the case of zone I, mitochondrial, myofibril and other volume densities were measured both with and without (Zone II) including the subsarcolemmal region (Fig. 1B).

4. The volume density of intermyofibrillar (V_v(mi.z)) and subsarcolemmal mitochondria (V_v(ms.z)).

5. The surface density of the mitochondrial outer membrane (S_v(mt.z)).

6. Mean free spacing of mitochondria (Λa).

Statistical analysis.

Determination of significant variation ($P \leq 0.05$) of parameters between species and between zones was carried out using the non-parametric Mann-Whitney test.

<u>RESULTS.</u>

<u>Muscle fibre diameters and capillary supply.</u>

The frequency distribution of muscle fibre diameters (Fig. 2) illustrates the similarity in fibre size of slow muscle fibres between species.

Morphometric parameters describing the capillary supply to muscle fibres are included in Table 2. Interspecific variations in capillary supply do not appear to be directly related to either blood oxygen carrying capacity or swimming activity. For example, largest interspecific variation in capillary density occurs between the two haemoglobinless species, *C. aceratus* ($J_V(c,f)$ = 479) and *C. gunnari* ($J_V(c,f)$ = 1524). However species exhibiting low capillary density have capillaries of relatively large cross-sectional area. Therefore capillary volume density ($V_V(c,f)$), an estimate of blood volume in the tissue at any one time, is similar between species (Table 2).

<u>Mitochondrial and muscle fibre composition.</u>

Estimates of the cristae surface densities of mitochondria within the intermyofibril region ($S_V(cr.mi)$) were significantly lower (P<0.01) in the two channicthyid species than in the red-blooded species (Table 3; Fig.3)

In addition, highest volume densities of mitochondria within the intermyofibril zone are found in the two channichthyid species, *C. gunnari* (0.51) and *C. aceratus* (0.49) (Table 3; Fig 4). *C. aceratus* fibres contain lower volume densities of myofibrils and higher quantities of

lipid and sarcoplasmic reticulum within the core of the muscle fibre than observed in *C. gunnari* (Table 3).

Although slightly lower, values of mitochondrial density in *P. breviceps* were not significantly different ($P \leq 0.05$) from those of the channichthyid species. Mitochondrial volume densities within the red-blooded antarctic species ranged from 0.45 in *P.breviceps* to 0.25 in *N. gibberifrons* (Table 3; Fig. 5). Quantitative values of fibre composition in *P. bolini* were similar to *N. gibberifrons*. However, myofibrils in *P. bolini* were ribbon-like in arrangement and distinct from those of notothenioids (Fig. 5).

Spacing of mitochondria within the intermyofibril zone, measured as mean free spacing (Λ_a) was closest in species with highest mitochondrial volume and surface densities. The closest spacing occurred in *C. gunnari* (1.32μm) and the greatest in *N. gibberifrons* (2.96μm) (Table 3).

A division between subsarcolemmal and intermyofibrillar regions exists in the slow muscle fibres of antarctic fish. The composition of whole muscle fibres was determined in *C. gunnari*, *P. breviceps* and *P. bolini* (Table 3). In all three species volume densities of intermyofibrillar mitochondria ($V_v(mi,f)$ = 0.31–0.23) make up a larger proportion of the total than do subsarcolemmal mitochondria ($V_v(ms,f)$ = 0.19–0.14).

Mitochondrial distribution within slow muscle fibres.

Variations in composition across the slow fibres of *C. aceratus*, *N. gibberifrons* and the temperate fish

Callionymus lyra, are summarised in Table 5. In all three species highest mitochondrial and lowest myofibrillar volume densities were found nearest the fibre border, in zone I (Fig 6). Further from the fibre border, within zone II, a dense band of myofibrils occurred, interspersed with significantly ($P \leq 0.05$) fewer mitochondria than in zone I as a whole.

A decrease in mitochondrial volume density towards the fibre centre, occurred in all three species. However in contrast to *N. gibberifrons* and *C. lyra*, in *C. aceratus* the decrease in mitochondrial volume density with distance from the fibre edge is not significant ($P \leq 0.05$). Mitochondrial volume density is as high as 0.38 even in the fibre centre, 35um from the sarcolemmal membrane (Fig. 4). Only slight variations in the surface density of the outer membrane ($S_v(mt.z)$) and mitochondrial spacing (Λa), were observed throughout the fibre cross-section in *C. aceratus* (Table 5; Fig 6).

Total volume densities of mitochondria are significantly lower in the temperate water species, *C. lyra* than in the notothenioids studied (Table 4). In addition, mitochondrial volume density declines more rapidly with increased distance from the fibre border in C. lyra. (Table 5. Fig. 6) The significant reduction in mitochondrial volume density towards the fibre centre in *N. gibberifrons* ($P \leq 0.01$) and *C. lyra* ($P \leq 0.001$) is correlated to increased spacing between mitochondria and an increased myofibril component (Fig. 6).

DISCUSSION.

This study illustrates that in the case of antarctic fish, muscle mitochondria do not appear to be invariant building blocks. Significantly lower values for cristae surface density (S_v(cr,mi)) were observed in the two channichthyid species, *C. gunnari* (25.2 μm^2 μm^{-3}) and *C. aceratus* (28.2 μm^2 μm^{-3}) than in the red-blooded species (32.2–37.0 μm^2 μm^{-3}) (Fig. 3).

The reactions of oxidative phosphorylation involve proteins either within the matrix (pyruvate dehydrogenase and most of the enzymes involved in the citric acid cycle) or embedded in the cristae, including cytochrome oxidase complex and the F_1/F_0 ATPase complex (see: Darnell *et al.*, 1986. for review). Assuming that the density of enzyme complexes is similar in the cristae of different species, cristae density can be related to the aerobic capacity of mitochondria. Therefore, the lower cristae density in channichthyids suggests lower enzyme concentrations, oxygen consumption and ATP production capacity per unit volume of mitochondria than in red-blooded species.

Structural estimates of low oxygen consumption in the mitochondria of channichthyids are consistent with biochemical evidence of aerobic capacity. For example, maximal activity of cytochrome oxidase in the slow muscle of *C. aceratus* (15.1 μmol substrate/g wet weight/min) are slightly lower than in *N. rossii* (19.9 μmol substrate/g wet weight/min), despite higher mitochondrial volume densities in *C. aceratus* (Johnston & Harrison, 1987). In addition, similar respiration rates per gram wet weight of muscle,

were found for slow muscle fibres isolated from *C. aceratus* and *N. gibberifrons* (Johnston, 1987).

Differences in enzyme activities associated with either acclimation of fish to varying temperatures (Tytler & Sidell, 1984) or different life-history stages (Eggington, 1986), result from alterations in mitochondrial volume densities rather than cristae structure. In the pigeon, *Columbia livia*, much higher cristae surface densities in type-1 fibres (31.4 $\mu m^2\ \mu m^{-3}$) than type-2 (19.7 $\mu m^2\ \mu m^{-3}$), are consistent with a thirty fold variation in enzyme activity (James & Meek, 1979). In contrast, mitochondria from fast and slow fibre types in goldfish, *Carassius auratus* (Tytler & Sidell, 1984) and american eel, *Anguilla rostrata*, (Eggington, 1986) contain similar cristae densities.

Mitochondrial distribution and spacing in relation to diffusion within muscle fibres.

It is thought that high concentrations of mitochondria occur close to the sarcolemma of mammalian skeletal muscle fibres because PO_2 is highest in this region but declines rapidly towards the fibre centre (Kayar *et al.* 1988). Alternatively, there is evidence that in contrast to Krogh type models, PO_2 gradients are shallow across mammalian muscle fibres. Direct measurements of oxygen pressure gradients across working muscle fibres have been made using myoglobin cryospectrophotometry (Gayeski & Honig, 1986). They suggest that there is low resistance to oxygen diffusion across mammalian slow muscle fibres, largely due

to the oxygen binding properties of myoglobin. Models of oxygen transfer predicting a sharp decline in PO_2 close to the sarcolemma (Krogh, 1919; Mainwood & Rakusan, 1982) appear to be inappropriate to fish muscle fibres. In all three species studied, including the temperate water species *C. lyra*, the decline in mitochondrial volume densities away from the fibre border is relatively slight (Fig. 6) in comparison with the mammalian situation. For example, in skeletal muscle fibres of the horse mitochondrial volume density decreases from 24-10% at the fibre border near to a capillary to 4-1% in the fibre core (Kayar *et al.* 1988).

Two points in particular suggest that oxygen transport across muscle fibres in antarctic fish is not a limiting factor affecting structural design. Channichthyid species which lack myoglobin in slow muscle fibres (Walesby *et al.* 1982), have elevated mitochondrial volume densities in comparison to red-blooded species (Tables 3 & 4). Secondly, the particularly homogeneous distribution of mitochondria across the slow fibres of *C. aceratus* (Figs. 4 & 6), also emphasises the fact that the distance between capillaries and mitochondria is not a constraint on fibre diameter.

There is a possibility that high mitochondrial volume densities may aid oxygen diffusion. Transport of oxygen may be facilitated along mitochondrial membranes, due to the higher solubility of oxygen in the lipid of membranes than in the aqueous cytoplasm (Bakeeva *et al.* 1978). However, this does not account for the homogeneous distribution of mitochondria. It is more likely that diffusion of molecules

between mitochondria and myofibrils is the more important determinant of fibre structure. High mitochondrial volume densities within the fibre core mean that mitochondria are in close proximity to myofibrils.

Mean free spacing (Λa) is an estimate of the average distance separating individual mitochondria. It therefore provides an indication of the diffusion path length between mitochondria. Reduction in temperature from 25° C to 5° C results in a 3.1-3.4 fold decrease in diffusivity constant of small molecules in the slow muscle cytoplasm of goldfish, *Carrasius auratus* (Sidell & Hazel, 1987). Following acclimation, an increase in mitochondrial volume density results in a compensatory reduction in mean free spacing from 11.8μm at 25° C to 2.9μm at 5° C (Tytler & Sidell, 1984). Values for mean free spacing in the slow fibres of antarctic fish are generally even lower than those in cold acclimated goldfish (Table 4). In addition, spacing between mitochondria remains small throughout the muscle fibres, especially in *C. aceratus* (Table 5). Elevated mitochondrial densities and their homogeneous distribution in antarctic fish, may be an adaptation to overcome diffusion limitations of molecules between mitochondria and myofibrils.

These findings question the functional significance of the concentration of mitochondria around the periphery of muscle fibres. Subsarcolemmal mitochondria may supply ATP to the plasma membrane, while intermyofibrillar mitochondria support the contractile elements. Variation in the respiratory characteristic of the two mitochondrial

populations may indicate their different functions (Jones, 1986). In addition, theoretical models have demonstrated that clustering of mitochiondria close to capillaries, may enhance oxygen transfer from capillaries to mitochondria (Mainwood & Rakusan, 1982).

If diffusion of oxygen is not a limitation, as discussed above, there remains the paradox of why channichthyids possess muscle fibres containing mitochondria with reduced aerobic capacity. Further studies are required to answer this problem.

Structure and function in the muscle fibres of fish.

The term symmorphosis describes a concept of regulated economical design (Taylor & Weibel, 1981). The principle proposes that structural design commensurate to functional needs should apply from cells and their organelles to whole organisms. In mammalian respiratory systems, mitochondrial volumes are directly matched to maximum oxygen consumption, demonstrating symmorphosis (Taylor *et al.* 1987). However, not all levels of structural organisation in mammalian systems exhibit symmorphosis directly. For example, the potential oxygen delivery by capillaries is not only related to the microvasculature but also involves blood oxygen carrying capacity (Taylor *et al.* 1987).

In antarctic fish, blood oxygen carrying capacity differs 10 fold between *C. aceratus* and red-blooded notothenioids (Holeton, 1970). Several factors are thought to compensate for the reduced oxygen carrying capacity in channichthyids. These include high blood volumes (Twelves,

1972), elevated cardiac output (Hemmingsen & Douglas, 1977) and large bore capillaries allowing fast blood flow rates (Everson, 1984; Fitch *et al*. 1984, Table 2). Obviously the deductions about oxygen transfer that can be made solely from studies of the structure of the microvasculature are limited.

Fish muscle exhibits a high level of plasticity in order to compensate for environmental change. Furthermore, acclimatory changes have been shown to alter capillary supply and mitochondrial volume density independently. For example, in crucian carp (*Carassius carassius*) capillary density is affected little by hypoxic conditions, whereas mitochondrial content of slow muscle increases 67% (Johnston & Bernard, 1983). European eels (*Anguilla anguilla*) acclimated to 10°C, exhibit increased mitochondrial densities but no observable alterations in capillary supply, compared to fish at 29°C (Eggington & Johnston, 1984). In addition, there are large interspecific variations in the form and degree of structural modification in response to environmental change. Such factors emphasise the complexity of design in fish muscle and the difficulties involved in relating structure to function.

Table 4.1. Standard lengths, ecology and normal temperature of the species studied.

Parameter	*C. aceratus*	*C. gunnari*	*N. gibberifrons*	*P. breviceps*	*P. bolini*	*C. lyra*
Standard length (mm) (mean ± S.E.)	528 ± 35	245 ± 7	311 ± 12	133 ± 11	59 ± 2	183 ± 8
Ecology	demersal	pelagic	demersal	pelagic	mesopelagic	demersal
Feeding habit	ambush [1] predator	50% krill [2,3] in diet	infauna[4] slurp feeder	krill in[5] diet	zooplankton[6] diurnal migrations	infauna[7]
temperature (°C)	-0.7	0.5 to 3.0	-0.7	0.5 to 3.0	-0.5 to 2.0	5 to 15

References

1. Daniels (1982).
2. Permitin & Taverdieva (1972).
3. Kock (1985).
4. Daniels (1982).
5. Andriashev (1987).
6. Torres & Somero (1988).
7. Lythgoe & Lythgoe (1971).

Table 4.2. Fibre size distribution and capillary supply to the slow muscle of five species of antarctic fish. (mean ± S.E.).

Parameter.	*C.aceratus*	*C.gunnari*	*N. gibberifrons*	*P.breviceps*	*P.bolini* (myotomal)
N(f)	136	200	162	200	287
â(f) (μm^2)	3900 ±200	1410 ±56	1880 ±110	1200 ±72	1010 ±43
d(f) (μm)	66.2 ±2.0	40.6 ±0.8	45.8 ±1.3	36.4 ±1.0	34.6 ±0.8
C:F	1.83	2.10	1.72	1.68	1.37
â(c) (μm^2)	79.9	21.6	35.9	27.1	18.0
b(c) (μm)	36.1	19.0	24.7	20.5	17.6
$N_A(c,f)$ (mm^{-2})	469	1494	917	1404	1290
$J_V(c,f)$ (mm^{-3})	479	1524	935	1432	1316
$V_V(c,f)$	0.038	0.033	0.034	0.039	0.024
$S_V(c,f)$	172.9	289.6	230.9	293.6	231.7

â(f), fibre area.
d(f), fibre diameter.
C:F, capillary to fibre ratio.
â(c), capillary cross sectional area.
b(c), capillary perimeter.
$N_A(c,f)$, capillary density per unit area of muscle fibre.
$J_V(c,f)$, capillary density per unit volume of muscle fibre.
$V_V(c,f)$, capillary volume density.
$S_V(c,f)$, capillary surface density.

Table 4.3. Mitochondrial distribution and composition within the interfibrillar zone of slow muscle fibres from five antarctic fish. (mean ± S.E.)

Parameter	*C. aceratus*	*C. gunnari*	*N. gibberifrons*	*P. breviceps*	*P. bolini* (myotomal)
$V_V(mi)$	0.49 ± 0.01	0.51 ± 0.02	0.25 ± 0.02	0.46 ± 0.02	0.30 ± 0.02
$V_V(mf)$	0.40 ± 0.02	0.48 ± 0.02	0.65 ± 0.02	0.51 ± 0.02	0.68 ± 0.02
$S_V(mi)$ ($\mu m^2 \mu m^{-3}$)	1.62 ± 0.06	2.01 ± 0.08	1.15 ± 0.08	1.96 ± 0.08	1.60 ± 0.06
Λa (um)	1.32 ± 0.06	1.04 ± 0.07	2.96 ± 0.25	1.18 ± 0.08	1.7 ± 0.09
$S_V(cr,mi)$ ($\mu m^2 \mu m^{-3}$)	28.2 ± 0.8	25.2 ± 1.5	37.0 ± 1.0	32.8 ± 1.3	32.2 ± 1.3

$V_V(mi)$, volume density of intermyofibrillar mitochondria.
$V_V(mf)$, volume density of myofibrils.
$S_V(mi)$, surface density of intermyofibrillar mitochondria.
a, mean free spacing of mitochondria.
$S_V(cr,i)$, cristae surface density.

Table 4.4. Composition of slow muscle fibres from three antarctic fish. (mean ± S.E.).

Parameter.	*C. gunnari*	*P. breviceps*	*P. bolini* (myotomal)
â(f).	1662 ± 186	1563 ± 203	1066 ± 43
V_v(mt,f)	0.49 ± 0.01	0.45 ± 0.02	0.36 ± 0.02
V_v(mi,f)	0.31 ± 0.01	0.25 ± 0.01	0.23 ± 0.01
V_v(ms,f)	0.18 ± 0.01	0.19 ± 0.01	0.14 ± 0.01
V_v(mf,f)	0.40 ± 0.01	0.48 ± 0.02	0.57 ± 0.02

â(f), fibre area.
V_v(mt,f), total mitochondrial volume density.
V_v(mi,f), volume density of intermyofibrillar mitochondria.
V_v(ms,f), volume density of subsarcolemmal mitochondria.
V_v(mf,f), volume density of myofibrils.

Table 4.5(A). Stereological parameters describing the composition of slow muscle fibres at varying distance from the fibre border, in the haemoglobinless/myoglobinless antarctic fish, *Chaenocephalus aceratus*. (mean ± S.E.).

Chaenocephalus aceratus:

	Position within muscle fibre. (distance from fibre border)			
	Zone I	Zone II	Zone III	Zone IV
Parameter	(0.0-6.75 um)	(2.0-6.75um)	(7.5-15.0um)	(15-35.0um)
V_v(mt.z)	0.43 ± 0.04	0.28 ± 0.03	0.38 ± 0.03	0.38 ± 0.03
V_v(mi.z)	0.11 ± 0.03			
V_v(ms.z)	0.32 ± 0.06			
V_v(mf.z)	0.28 ± 0.05	0.61 ± 0.04	0.53 ± 0.04	0.51 ± 0.03
V_v(other.z)	0.30 ± 0.03	0.12 ± 0.03	0.11 ± 0.02	0.11 ± 0.02
S_v(mt.z) ($\mu m^2 \mu m^{-3}$)	2.10 ± 0.11		1.72 ± 0.13	1.78 ± 0.16
a(mt.z) (um)	1.16 ± 0.13		1.60 ± 0.17	1.54 ± 0.19

V_v(mt.z) mitochondrial volume density in zone.
V_v(mi.z) intermyofibrillar mitochondrial volume density in zone.
V_v(ms.z) subsarcolemmal mitochondrial volume density in zone.
V_v(mf.z) myofibrillar volume density in zone.
V_v(other.z) volume density of remaining constituents in zone.
S_v(mt.z) surface density of outer mitochondrial membrane in zone
a(mt.z) mean free spacing of mitochondria in zone.

Table 4.5(B). Stereological parameters describing the composition of slow muscle fibres at varying distance from the fibre border, in the red-blooded antarctic fish *Notothenia gibberifrons*. (mean ± S.E.).

Notothenia gibberifrons:

	Position within muscle fibre. (Distance from fibre border)			
	Zone I	Zone II	Zone III	Zone IV
Parameter	(0.0-6.75 um)	(2.0-6.75um)	(7.5-15.0um)	(15-35.0um)
V_v(mt.z)	0.41 ± 0.04	0.16 ± 0.03	0.30 ± 0.02	0.28 ± 0.01
V_v(mi.z)	0.12 ± 0.03			
V_v(ms.z)	0.29 ± 0.05			
V_v(mf.z)	0.37 ± 0.05	0.74 ± 0.03	0.58 ± 0.02	0.60 ± 0.01
V_v(other.z)	0.22 ± 0.04	0.10 ± 0.02	0.12 ± 0.01	0.12 ± 0.02
S_v(mt.z) ($\mu m^2 \mu m^{-3}$)	1.90 ± 0.17		1.56 ± 0.12	1.37 ± 0.10
a(mt.z) (μm)	1.48 ± 0.23		1.96 ± 0.21	2.22 ± 0.22

V_v(mt.z) mitochondrial volume density in zone.
V_v(mi.z) intermyofibrillar mitochondrial volume density in zone.
V_v(ms.z) subsarcolemmal mitochondrial volume density in zone.
V_v(mf.z) myofibrillar volume density in zone.
V_v(other.z) volume density of remaining constituents in zone.
S_v(mt.z) surface density of outer mitochondrial membrane in zone
a(mt.z) mean free spacing of mitochondria in zone.

Table 4.5(C). Stereological parameters describing the composition of slow muscle fibres at varying distance from the fibre border, in the temperate water fish, *Callionymus lyra*. (mean ± S.E.).

Callionymus lyra:

Parameter	Position within muscle fibre. (distance from fibre border). Zone I (0.0-6.75 um)	Zone II (2.0-6.75um)	Zone III (7.0-20.0 um)
V_v(mt.z)	0.34 ± 0.02	0.20 ± 0.01	0.22 ± 0.02
V_v(mi.z)	0.13 ± 0.01		
V_v(ms.z)	0.21 ± 0.02		
V_v(mf.z)	0.51 ± 0.02	0.73 ± 0.02	0.67 ± 0.02
V_v(other.z)	0.14 ± 0.01	0.08 ± 0.01	0.10 ± 0.01
S_v(mt.z)	2.04 ± 0.08		1.38 ± 0.09
a(mt.z)	1.35 ± 0.08		2.40 ± 0.17

V_v(mt.z) mitochondrial volume density in zone.
V_v(mi.z) intermyofibrillar mitochondrial volume density in zone.
V_v(ms.z) subsarcolemmal mitochondrial volume density in zone.
V_v(mf.z) myofibrillar volume density in zone.
V_v(other.z) volume density of remaining constituents in zone.
S_v(mt.z) surface density of outer mitochondrial membrane in zone
a(mt.z) mean free spacing of mitochondria in zone.

Table 4.6. Comparative data on the capillary supply and mitochondrial content of the slow muscle that powers sustained swimming, in fish species from differing environments and of varied activity level.

Species (Source)	Environment	Activity	N_A (c,f)	a(c)	V_v (c,f)	V_v (mt,f)
Notothenia rossii (1)	antarctic	2° pelagic				0.38
Notothenia neglecta (adult) (2)	antarctic	demersal	498	58.1	0.029	0.34
Pleuragramma antarcticum (3)	antarctic	pelagic	1057	28.4	0.031	0.56
Bullrout *Myoxoscephalus scorpius* (4)	cool temperate	demersal				0.23
Conger eel *Conger conger* (5)	cool temperate	demersal (anguilliform)	615	26	0.016	0.23
American eel *Anguilla rostrata* (6)	temperate	migratory (anguilliform)	1869	14.5	0.028	0.22
	temperate	non-migratory	1363	13.7	0.019	0.16
Tench *Tinca tinca* (7)	temperate	freshwater relatively sedentary	2672	22	0.059	0.23
Crucian carp *Carrasius carrasius* (8)	temperate	freshwater sedentary	1639	20	0.034	
Hatchetfish *Argyropelecus hemigymnus* (9)	temperate	mesopelagic	2994			0.44
Anchovy *Engraulis encrasiocolus* (10)	warm temperate	pelagic	6000			0.46
Skipjack tuna *Katsuwonus pelamis* (11)	tropical	pelagic				0.38

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1. Walesby & Johnston.(1980)
2. Johnston & Camm.(1987).
3. Johnston *et al*. (1988).
4. Johnston (1987). (PTO).

5. Eggington (1982).
6. Eggington (1986).
7. Johnston & Bernard (1982 & unpublished data)
8. Johnston & Bernard (1984).
9. Salamonski & Johnston (1984).
10. Johnston (1982).
11. Hochachka *et al.* (1978)

Figure 4.1.

Diagram of a slow muscle fibre from *Notothenia gibberifrons*, illustrating the sampling locations (zones I, III & IV) for measurement of mitochondrial distribution across fibres. Zone II consisted of the intermyofibrillar region within zone I. Each zone is 7.5µm in width.

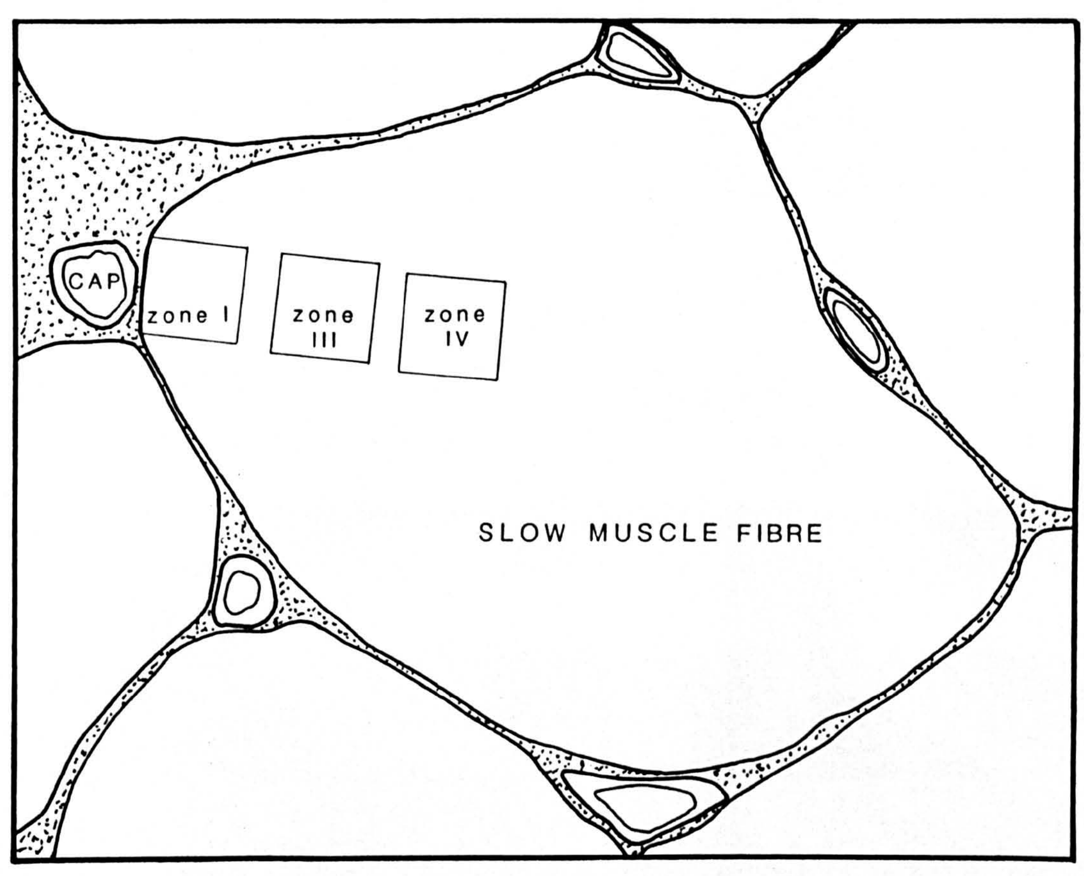
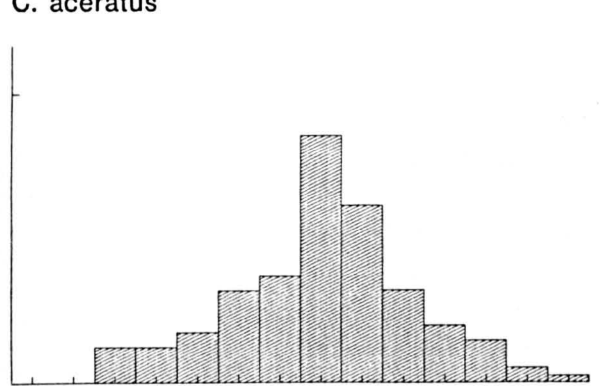
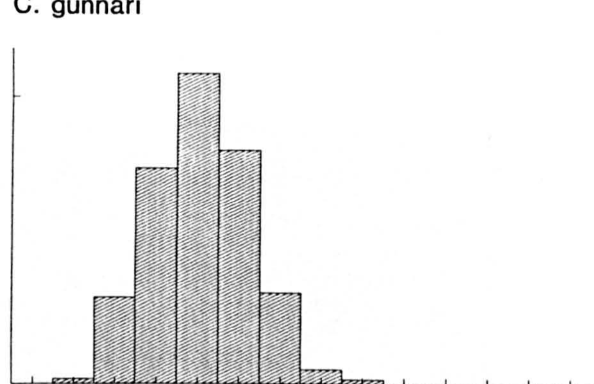
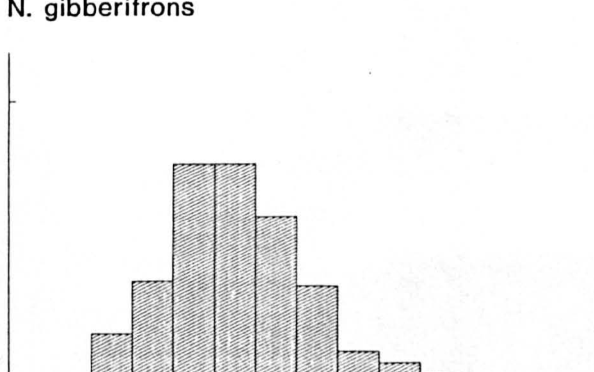

CAP
zone I
zone III
zone IV
SLOW MUSCLE FIBRE

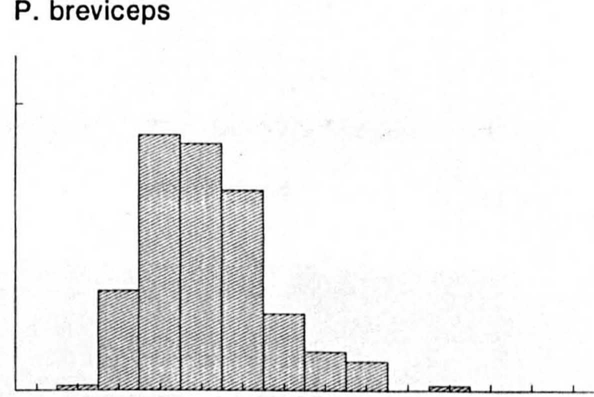
Figure 4.2.

Histograms showing the frequency distribution of diameter of the slow muscle fibres from five antarctic teleosts. (see Table 3.2 for sample size and mean ± S.E. values)


C. aceratus


C. gunnari


N. gibberifrons


P. breviceps

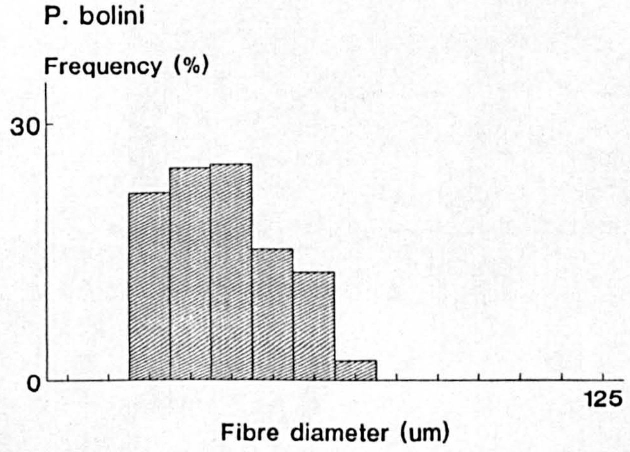

P. bolini
Frequency (%)
30
0
125
Fibre diameter (um)

Figure 4.3.

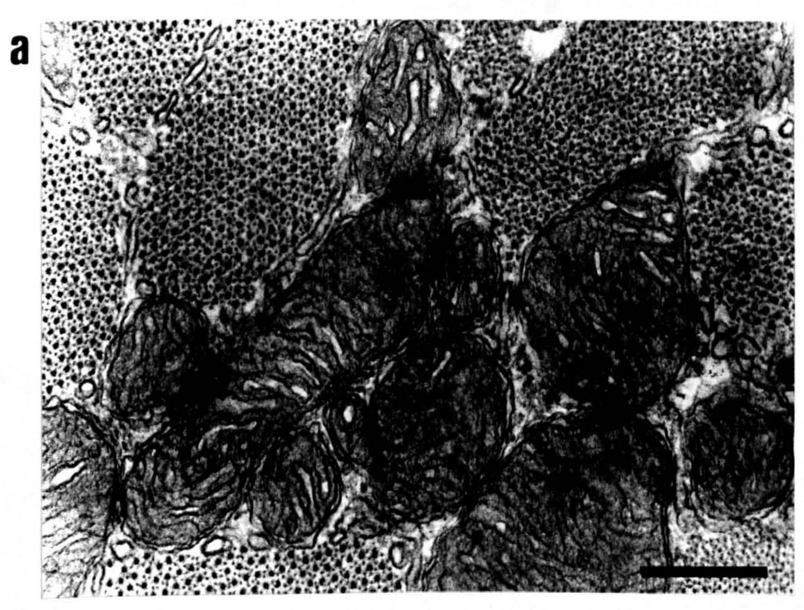
Electron micrographs of intermyofibrillar mitochondria from the slow muscle of two antarctic fish. The difference in cristae surface density between red-blooded and channichthyid species is illustrated.

(a). *Notothenia gibberifrons*: mitochondria with densely packed cristae.

(b). *Champsocephalus gunnari*: illustrating the sparse cristae density.

Scale bars = 0.4µm.

a



b

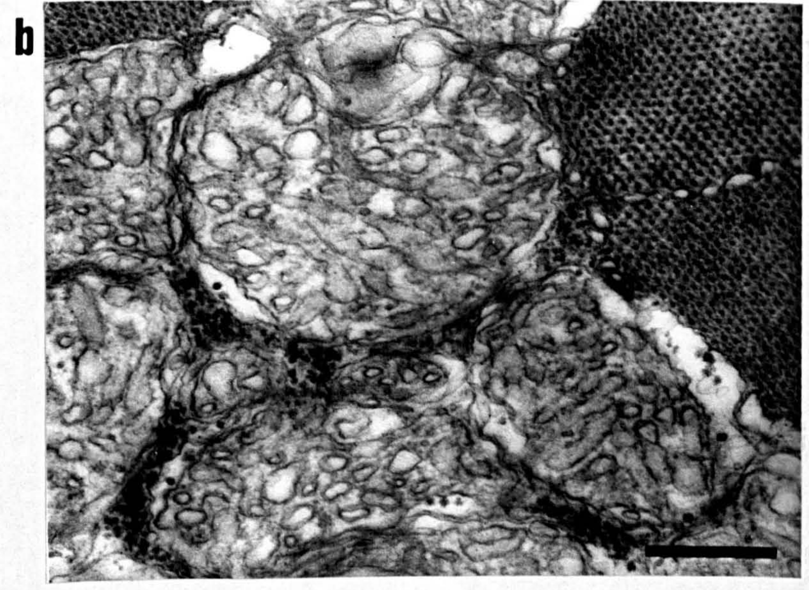


Figure 4.4.

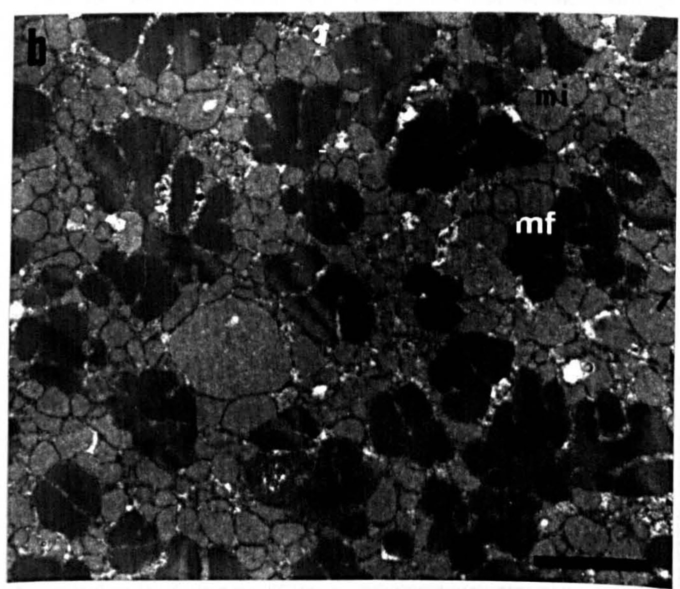
Electron micrographs of the slow muscle from two channichthyid species.

(a). *Chaenocephalus aceratus*: illustrating the very high mitochondrial volume densitites sometimes found in subsarcolemmal regions (ms subsarcolemmal mitochondria; mf myofibril; L lipid deposits; N nucleus). Scale bar = 4µm

(b). *Chaenocephalus aceratus*: note the relatively high mitochondrial volume density within the core of the muscle fibre (mi = intermyofibrillar mitochondria; mf myofibril). Scale bar = 3µm.

(c). *Chamsocephalus gunnari*: the high densities of both subsarcolemmal and intermyofibrillar mitochondria are illustrated (mf myofibril; mi intermyofibrillar mitochondria; ms subsarcolemmal mitochondria). Scale bar = 2µm.

a
ms
L
N


b
mi
mf

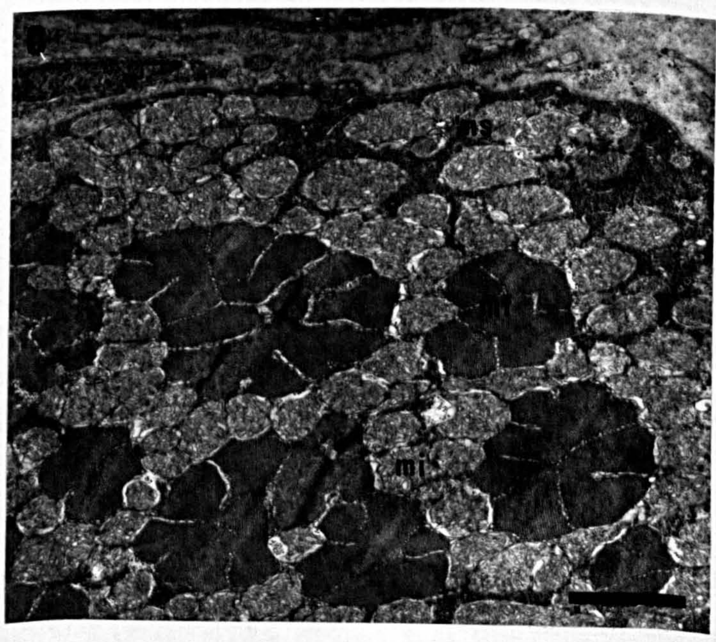

ms
mi

Figure 4.5.

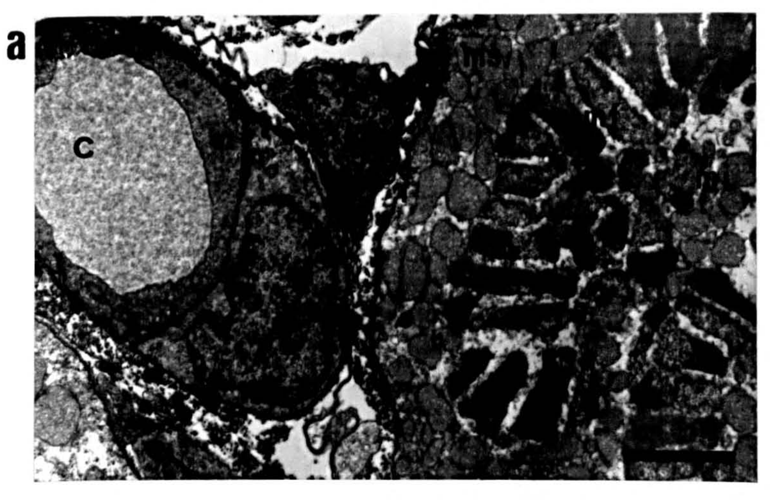
Electron micrographs of the slow muscle fibres of three red-blooded antarctic species.

(a). *Notothenia gibberifrons*: micrograph showing the distribution of mitochondria and myofibrils in relation to a capillary (c capillary; mf myofibril; mi intermyofibrillar mitochondria; ms subsarcolemmal mitochondria). Scale bar = 2µm.

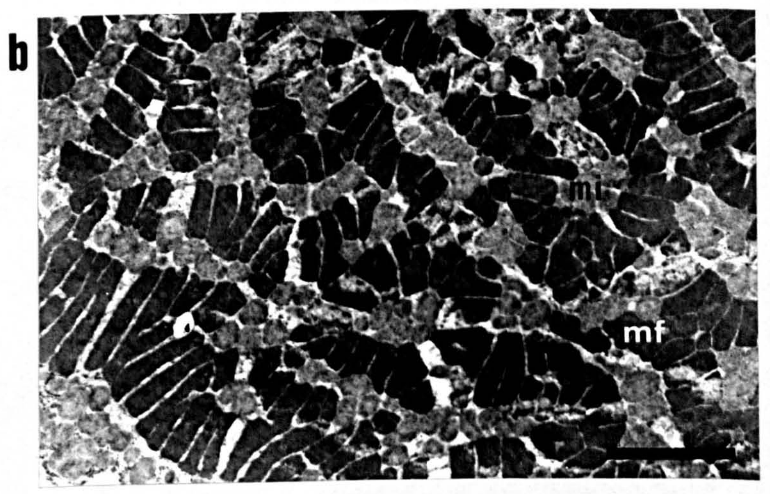
(b). *Protomyctophum bolini*: intermyofibrillar region (mf myofibril; mi intermyofibrillar mitochondria). Scale bar = 4µm.

(c). *Psilodraco breviceps*: an active species with relatively high densities of mitochondria (mf myofibril; mi intermyofibrillar mitochondria). Scale bar = 2 µm.

a



b



c

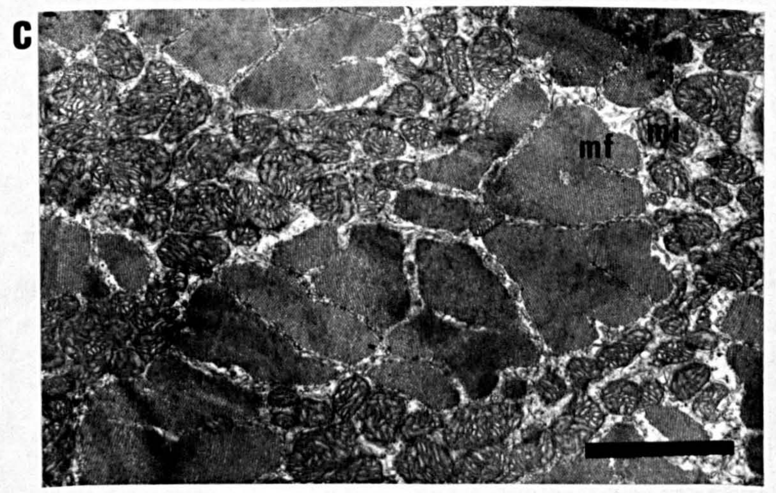
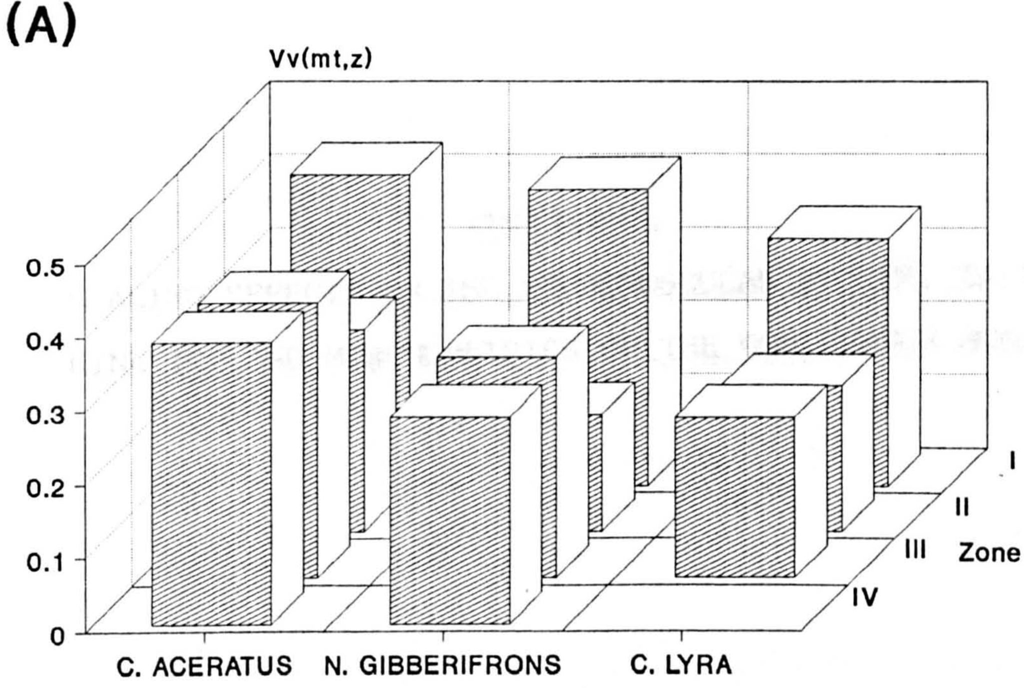
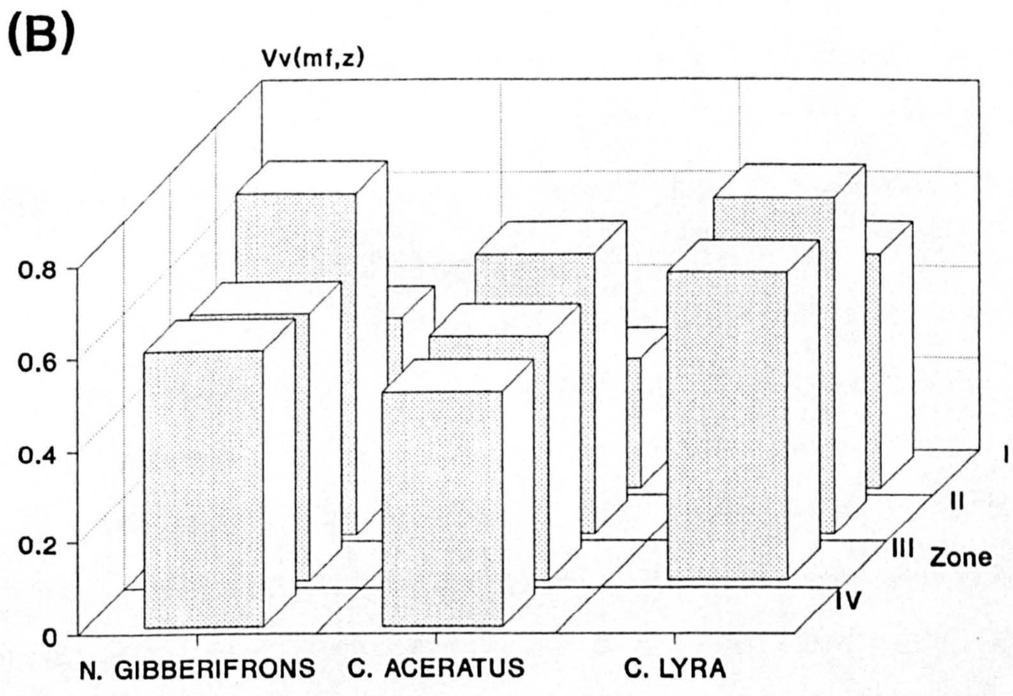


Figure 4.6.

Diagrams representing the pattern of distribution of (A) mitochondria and (B) myofibrils across the slow muscle fibres of *Chaenocephalus aceratus*, *Notothenia gibberifrons* and *Callionymus lyra*. (means ± S.E. are included in Table 5). Zone I is a region closest to the fibre border and adjacent to a capillary; zone IV is in the fibre centre.


(A)
Vv(mt,z)
0.5
0.4
0.3
0.2
0.1
0
C. ACERATUS
N. GIBBERIFRONS
C. LYRA
I
II
III
IV
Zone


(B)
Vv(mf,z)
0.8
0.6
0.4
0.2
0
N. GIBBERIFRONS
C. ACERATUS
C. LYRA
I
II
III
IV
Zone

CHAPTER 5.

SCALING EFFECTS ON THE NEUROMUSCULAR SYSTEM, TWITCH KINETICS AND MORPHOMETRICS OF THE COD, *GADUS MORHUA*

INTRODUCTION.

The fast muscle fibres in most vertebrates are focally innervated, with one or two motor axons terminating at a single endplate (Hess, 1970; Morgan & Proske, 1984;). In contrast, fast muscle in advanced teleosts has a distributed pattern of innervation, with numerous endplates terminating along the length of each fibre (Bone, 1964; Hudson, 1969; Altringham & Johnston, 1981). There would appear to be high selective pressure for the evolution of distributed innervation, since there is evidence of it's independent origin in several orders of teleosts (Ono, 1983).

Despite recent studies of the morphology and physiology of polyneuronal innervation (Westerfield *et al.* 1986; Altringham & Johnston, 1988b; Mos *et al.* 1988) , it's functional significance remains obscure. The pattern of innervation of the fast motor system has been correlated with the threshold swimming speeds for recruitment of different fibre types (Bone, 1978; Johnston, 1981). In contrast to elasmobranchs and primitive teleosts, many species exhibiting a distributed innervation, recruit fast muscle during low speed sustained swimming (Hudson, 1973; Johnston *et al.* 1977; Bone *et al.* 1978; Johnston & Moon, 1980a, 1980b; Rome *et al*, 1984). In addition, capillary and mitochondrial volume densities are significantly higher in fast muscle fibres with polyneuronal innervation compared to those with a focal pattern (Johnston & Moon, 1981; Johnston *et al.* 1983). This suggested that a distributed innervation pattern may possibly allow a graded response in

the fast muscle of higher teleosts . However, the mechanical properties of isolated focally and polyneuronally innervated fast muscle fibres are similar (Altringham & Johnston, 1988). Fast fibres from both *Myoxocephalus scorpius* and *Anguilla anguilla*, displayed all-or-none twitches in response to supra-threshold stimulii (Altringham & Johnston, 1987, 1988a).

As fish grow, the neuromuscular system must change to meet the new physiological and hydrodynamic conditions imposed by their increased size. Alterations in swimming kinematics and performance with size have been discussed previously (Chapters 1 & 3). In particular, variation in tail beat frequency and specific propulsive wavelength would be expected to alter the pattern of activation of muscle fibres. Even a superficial examination of fish of different sizes suggests that the number of endplates per fibre increases as fish grow.

This study includes examination of the size related changes in morphometrics, twitch kinetics and innervation pattern in the cod, *Gadus morhua*. The results are discussed with reference to the general problems of scaling in fish locomotion, and the functional significance of polyneuronal innervation.

MATERIALS AND METHODS.

Fish and morphometics.

Cod, *Gadus morhua*, were caught in the Firth of Forth between October and December 1988, and kept in the laboratory in flow through aquaria at 10-12° C for up to two weeks before use. Fish ranged in size from 9 to 45cm total length. Fish were killed by a blow to the head and pithed. After total length and weight were .recorded taken, the mechanical preparation was first removed. The length of the *myotome* was measured from the cleithrum to the base of the hypural complex (italisised myotome refers to sum of all myotomes). Tracings of the myotomal muscle cross section were made at 5 equal intervals along the *myotome*. *Myotomal* area was measured using a digitier pad interfaced to a microcomputer (Hewlett Packard). and an average value for muscle cross-sectional area determined. All the myotomal muscle from one side of the fish was then dissected and weighed to obtain a value for *myotomal* muscle weight.

Mechanics.

Twitch contraction parameters of fast fibres were recorded from a nerve-muscle preparation of abdominal myotomes. The abdomenal cavity of the fish was opened along the ventral midline, and a rectangular block of tissue removed. This included the 6 myotomes behind the insertion of the pectoral fins, and the full depth of the abdominal cavity. No slow fibres are located in this region. The preparation was pinned out in a temperature controlled bath, and immersed in Ringer solution of the following

composition: (mmol l^{-1}): 132.2 NaCl, 10 Na pyruvate, 2.6 KCl, 1 $MgCl_2$, 18.5 $NaHCO_3$, 3.2 NaH_2PO_4, 2.7 $CaCl_2$, pH 7.4 at 12° C. The fourth spinal nerve from the rostral end was drawn into a suction electrode, and single stimuli yeilding a maximal force response were given. Muscle displacement was recorded by resting a strain gauge (AE801, AME, Horten, Norway) on the surface of the myotome immediately rostral to the stimulated nerve. This method gave highly reproducible measurements of contraction kinetics in the intact myotome, independent of the positioning of the strain gauge. However, the system could not be calibrated for force generation by the muscle, since although the strain gauge was arranged to record in a plane parallel to the longitudinal axis of the fibres, some of the fibres in adjacent myotomes also contracted. Experiments were performed at 12° C and the time interval between the onset of force generation and the 90% relaxation point was measured. Beyond 90% relaxation, force decline is very slow. The results were highly reproducible, and the data from each preparation (minimum of 4 contractions) were meaned.

Histochemistry.

At the end of the mechanical experiments, the peritoneum over the studied myotome was removed, and the preparation was pinned out on silicone/elastomer (Sylgard 184, Dow Corning). Preterminal axons and neuromuscular junctions were stained using the acetylthiocholine technique of Naik (1963). After fixation for 2h at room

temperature in 10% formalin in 0.1M acetate buffer (pH 5.2), the preparation was washed in distilled water for 1h. Fibre bundles, approximately 1mm² were removed an fixed to elastomer strips. These were incubated in the acetylthiocholine solution at 37° C for 6-8h, or overnight at room temperature. After staining in ammonium sulphide, washing and clearing in glycerol, small bundles of 1-6 fibres were dissected and mounted in glycerol. The number of endplates on each fibre was counted using a Nikon Labophot microscope. The length of each fibre was determined using the microscope stage micrometer. In addition, the diameters of 50 fibres from each of 9 fish (9 - 41 cm) were determined.

RESULTS.

Mechanics.

In Fig. 1a, log. contraction time (t, ms) is plotted against the log. of total length (L, cm) of the fish. Contraction time is relatively independent of fish length, $t = 13.2\ L^{0.29}$ (Table 1). Representative force/time displacement records are shown in Fig. 1b, from 2 fish at the extremes of the size range.

Morphometrics.

In Fig.2, mean *myotomal* area, total/*myotomal* weight and mean muscle fibre/*myotomal* length, are plotted against total length (L) on log./log. plots. As expected, all show a marked dependence on L. *Myotomal* area increases with the

square of the length ($\alpha\ L^{2.05}$, Fig. 2a), and *myotomal* and total weight as the cube of total length ($\alpha\ L^{3.04}$ and $L^{3.08}$ respectively, Fig. 2b). Fibre and *myotomal* length (Fig. 2c) increase essentially linearly with fish length ($\alpha\ L^{1.08}$ and $L^{0.96}$).

Histochemistry.

The complexity of the polyneuronal innervation of fast muscle fibres of *Gadus morhua* is illustrated in Fig. 4. The number of endplates terminating on 10-40 fibres were counted for each fish. The number of endplates per fibre increased with increasing fibre length, from around 10 on fibres 2mm in length (~10cm fish) to 20 on 10mm fibres (~40 cm fish) (Fig.3a). However, mean spacing between endplates increased from around 0.25mm to 0.5mm (Fig. 3b). Mean endplate spacing was approximately linearly related to mean fibre diameter, the latter increasing approximately two fold over the size range studied (Fig. 3c). Endplate structure is shown in Fig. 5, from two fish of very different sizes.

DISCUSSION.

Muscle and locomotion.

Wardle (1975) measured the isotonic contraction time of myotomal blocks from a number of fish species, over a wide range of sizes. He found that contraction time increased as fish size increased, and suggested that contraction time could be used to predict maximum tailbeat frequency and hence maximum swimming speed. However, contraction time was defined as the time taken to reach peak tension, and did not include relaxation. Before the contracting muscle on one side can begin to bend the fish in that direction, the force generated must exceed that of the relaxing muscle on the contralateral side. It would therefore be more appropriate to include relaxation in twitch contraction time if it is to be used to predict maximum tailbeat frequency. This study has measured contraction time by a different method to that used by Wardle, and included most of the relaxation phase, yet obtain similar contraction times for the cod, and find a similar correlation with fish length. The isotonic preparation used by Wardle must have contracted more slowly than the near isometric fibres of our study, possibly due to the presence of a high proportion of damaged fibres in the excised myotomal blocks.

Measurement of tail-beat period in trout during fast starts (Webb, 1976) and short duration bursts (Bainbridge, 1958) were proportional to $L^{0.28}$ and $L^{0.18}$ respectively. Contraction time in cod is also relatively independant of size, scaling in proportion to $L^{0.29}$ (Fig. 1). Fast starts

appear to involve the recruitment of all the myotomal muscle on one side of the body simultaneously (Johnsrude & Webb, 1985). Therefore, measurement of twitch contraction times may give some indication of the duration of stages in the fast-start reaction. However, the extent to which contraction time determines maximum tail-beat frequency and hence maximum swimming speed is not clear. A maximum tail-beat frequency of 18 Hz has recently been recorded for mackerel during burst swimming (Wardle & He, 1988). In comparison, a maximum of 19Hz was predicted from muscle contraction times (i.e. time to peak tension). Studies on isolated preparations do not, however, take into account the complex myotomal geometry (Alexander, 1969). In an attempt to overcome this, Webb and Johnsrude (1988) studied the mechanical properties of one entire side of a fish, complete with skeletal elements (defined as the half-myotome). They conclude that other factors must play an important role, and suggest that in larger fish, temporal summation of twitches is needed to provide sufficient force for acceleration. The situation is almost certainly more complex still, since the muscle fibres are undergoing cyclical length changes during swimming. The phase relation between muscle length change and stimulation will greatly affect both the appparent contraction time and force generation (Josephson, 1985; Stevens, 1988).

Swimming performance will also be greatly influenced by size related changes in the geometry and proportion of myotomal muscle. Both *myotomal* and fibre length scale isometrically with total length (Fig. 2), but there is no

information on possible changes in fibre arrangement within individual myotomes. Such changes could influence both the time course and absolute force generated. Mean *myotomal* cross-sectional area and *myotomal* weight change geometrically with size (α $L^{2.05}$ and $L^{3.08}$ respectively; Fig. 2), as reported for the trout (Webb and Johnsrude, 1988). Geometric scaling of myotomal mass over a similar length range has also been reported in dace, goldfish (Bainbridge, 1960) and sockeye salmon (Webb, 1978). In the half-myotomal preparation studied by Webb and Johnsrude (1988), maximum power output scaled at a similar rate to muscle mass. Since force is proportional to cross-sectional area, we might expect it to scale geometrically too. However, Webb and Johnsrude (1988) found force was proportional to $L^{2.6}$ rather than $L^{1.8}$. In addition to possible changes in fibre geometry and the proportion of non-contractile material discussed by Webb and Johnsrude (1988), this result might be explained by changes in the intrinsic properties of the contractile proteins themselves. For instance, myofibrillar ATPase activity of fast muscle samples declines with length in a number of marine teleosts (Witthames & Greer-Walker, 1982).

Innervation.

As fish size, and the diameter and length of individual muscle fibres increase, the number of endplates terminating on each fibre also increases. However, the spacing between endplates also increases (Figs. 3 & 5). These results confirm those reported very recently by Mos *et al.* (1988) on several teleost species. Mos *et al.* (1988) argue that multilply innervated teleost fast fibres have only a limited ability to generate propagated action potentials, and that a distributed innervation is therefore necessary for the development of full mechanical activity through local depolarisation.

More recent studies of the properties of multiply innervated teleost muscle fibres do not support the concept of activation by local depolarisation proposed by Mos *et al.* (1988). Fast fibres consistently respond to single stimuli through a spinal nerve with action potentials, which generate mechanical twitches (Westerfield *et al.* 1986; Altringham & Johnston, 1988a; 1988b). In this respect they are similar to the focally innervated fast fibres present in primitive teleosts, elasmobranchs and other vertebrate muscles (Bone, 1964; Johnston, 1981). Since an all-or-none contraction is probably generated by an action potential in only one axon, and the muscle action potential is propagated down the fibre (Hudson, 1969; Altringham & Johnston, 1988b; 1989), the functional significance of this pattern of innervation remains obscure.

One possibility is that depolarisation may not be adequate to evoke action potentials at all synapses, and

multi-terminal innervation may ensure an all-or-none response to stimulation. As multiply innervated skeletal muscle from the frog (*Xenopus laevis*) is cooled, the proportion of fibres responding to a single nerve stimulus with subthreshold endplate potentials increases dramatically from 6% at 10-30°C to 60% at 2.5°C (Adams, 1989). However, even at 10-30°C, some synapses respond only with subthreshold potentials, but these are usually interrupted by an action potential propagated from another synapse on the fibre. Multi-terminal innervation, and the thermal independence of transmitter release over a wide temperature range may be of functional significance to poikilotherms which experience large variations in body temperature (Adams, 1989).

Mos *et al.* (1988) suggest that the distance over which a depolarisation attenuates is described by the space constant between individual end plates. As fibre diameter increases, the space constant of the membrane increases. The increase in the number of endplates is proposed to be the minimum required to maintain full activation of the muscle as the space constant increases, ensuring that no energy is wasted in supporting redundant synapses. This is in agreement with their finding that the spacing between endplates $\alpha \sqrt{\text{fibre diameter}}$, since the space constant $\alpha \sqrt{\text{fibre diameter}}$. In contrast, endplate spacing in *Gadus morhua* is linearly related to fibre diameter (Fig. 3c). However, too much emphasis should not be placed on this relationship, since the comparison is between mean values, and both parameters vary considerably within a single fish.

In addition, endplate spacing is variable along individual muscle fibres (Fig. 5). Furthermore, as a single axon can generate an action potential, the relevant space constant will be between endplates derived from the same axon rather than between adjacent terminals.

Suggestions as to why there has been such an apparently strong selective pressure for the evolution of polyneuronal innervation in teleosts (Bone & Ono, 1982) are rather more speculative. In insect flight muscle, multiple stimuli per oscillatory work cycle increase power output above that generated from single twitches (Josephson, 1985). The myotomal muscle of a swimming fish also undergoes periodic length-change and stimulus cycles during locomotion (Grillner & Kashin, 1976; Hess & Videler, 1984). Polyneuronal innervation may be part of a mechanism for changing the number of stimuli per work cycle, optimising power output over a range of swimming speeds (Johnston & Altringham, 1988).

Similarly, the need to make new endplates on lengthening fibres is difficult to explain if this also involves innervation by new motoneurons. In adult sculpin, *Myoxocephalus scorpius*, a typical fast fibre is innervated by around 4-6 axons (Altringham & Johnston, 1989) and pre-terminal branching is presumed to account for the 8-20 endplates which Hudson (1969) counted on these fibres. A very similar arrangement has been found in the zebrafish by Westerfield et al. (1986). Evidence suggests that the large number of endplates on cod fast fibres also arise from the preterminal branching of a rather smaller number of axons

(Altringham & Johnston, 1981; Egginton & Johnston, 1986). In the zebrafish, all the muscle fibres of a myotome are innervated by just three individually identifiable primary motoneurons, in larvae and adults. These innervate mutually exclusive regions of the muscle, and make endplates at multiple sites along the length of each fibre. Each fibre is innervated by one primary and several secondary motoneurons. The origins and innervatory patterns of the secondary motoneurons are less well understood, although in some cases they originate in adjacent myotomes. During growth, terminations on new or lengthening fibres could arise by further preterminal branching of primary or secondary motoneurons, or through innervation by additional axons. Since Westerfield *et al.* (1986) found that the number of primary motoneurons did not increase from larval to adult stages, the mechanism must be restricted to branching of existing motoneurons, and/or additional secondary motoneurons. Less is known about other teleost species, so it is difficult to say how general this pattern is, and therefore how applicable to the cod. However, as suggested by Westerfield *et al.* (1986), whatever the mechanism, it is probably more complex than the system of general competition among motoneurons found in focally innervated mammallian twitch fibres (Van Essen, 1982), and must involve some ability to discriminate between different classes of motor axon.

Table 5.1. Allometric equations of the scaling relationship of morphometric and contractile parameters in cod, *Gadus morhua*. (Parameter = *a* * Total length ^{b})

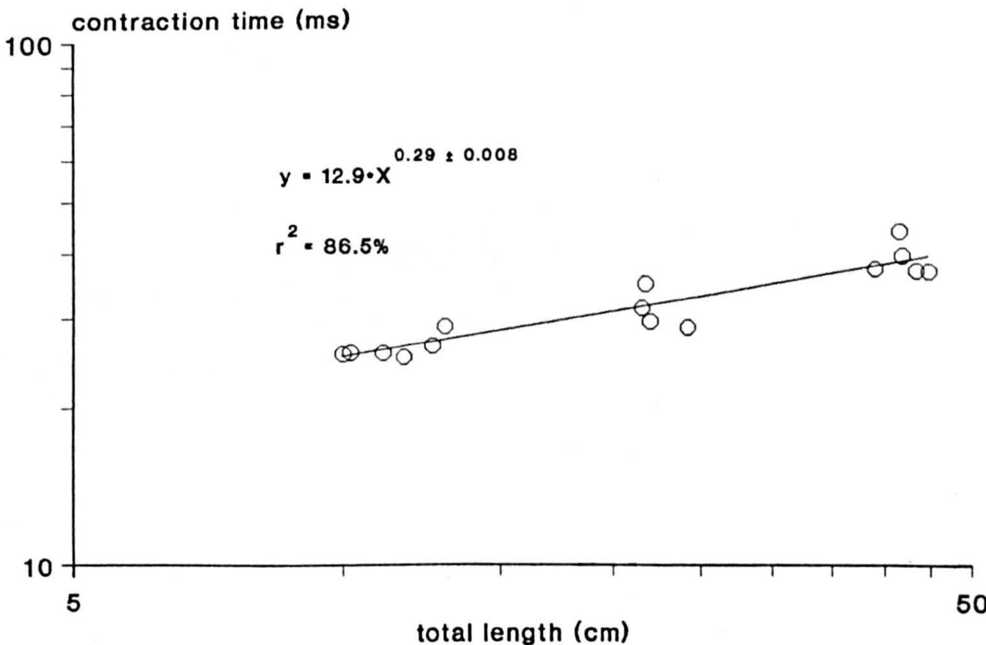
Parameter	Coefficient *a*	coefficient *b*	r^2 (%)
Myotomal area.	0.006 ± 0.000	2.06 ± 0.01	98.8
Myotomal length	0.721 ± 0.010	0.96 ± 0.004	99.6
Myotomal weight	0.003 ± 0.000	3.08 ± 0.02	98.5
Total weight	0.007 ± 0.000	3.04 ± 0.01	99.4
Fibre length	0.156 ± 0.020	1.08 ± 0.02	95.8
Contraction time	12.88 ± 0.32	0.29 ± 0.09	86.5

Figure 5.1.

The scaling relationship of contraction time of myotomal fast muscle in the cod, *Gadus morhua*.

(a). Allometric plot of twitch contraction time (time to 90% relaxation) to fish total length.

(b). Force/time displacement records of twitch contraction of fast muscle from two different sized cod: (A) Total length 440mm. t_{90} = 36ms. (B) Total length 91mm. t_{90} = 25ms.



(b)

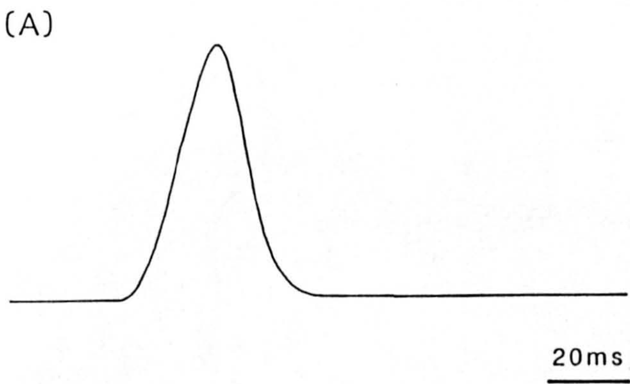


Figure 5.2.

Allometric scaling relationships of the morphometric parameters of cod, *Gadus morhua*. The following parameters are scaled in relation to fish total length:

(a). Mean cross sectional area of the myotomal muscle, measured at five equidistant points along the *myotome*.

(b). Total weight and *myotomal* muscle weight

(c). Fast muscle fibre length (mean values of 50 fibres per fish).

(Full regression equations are given in Table 5.1).

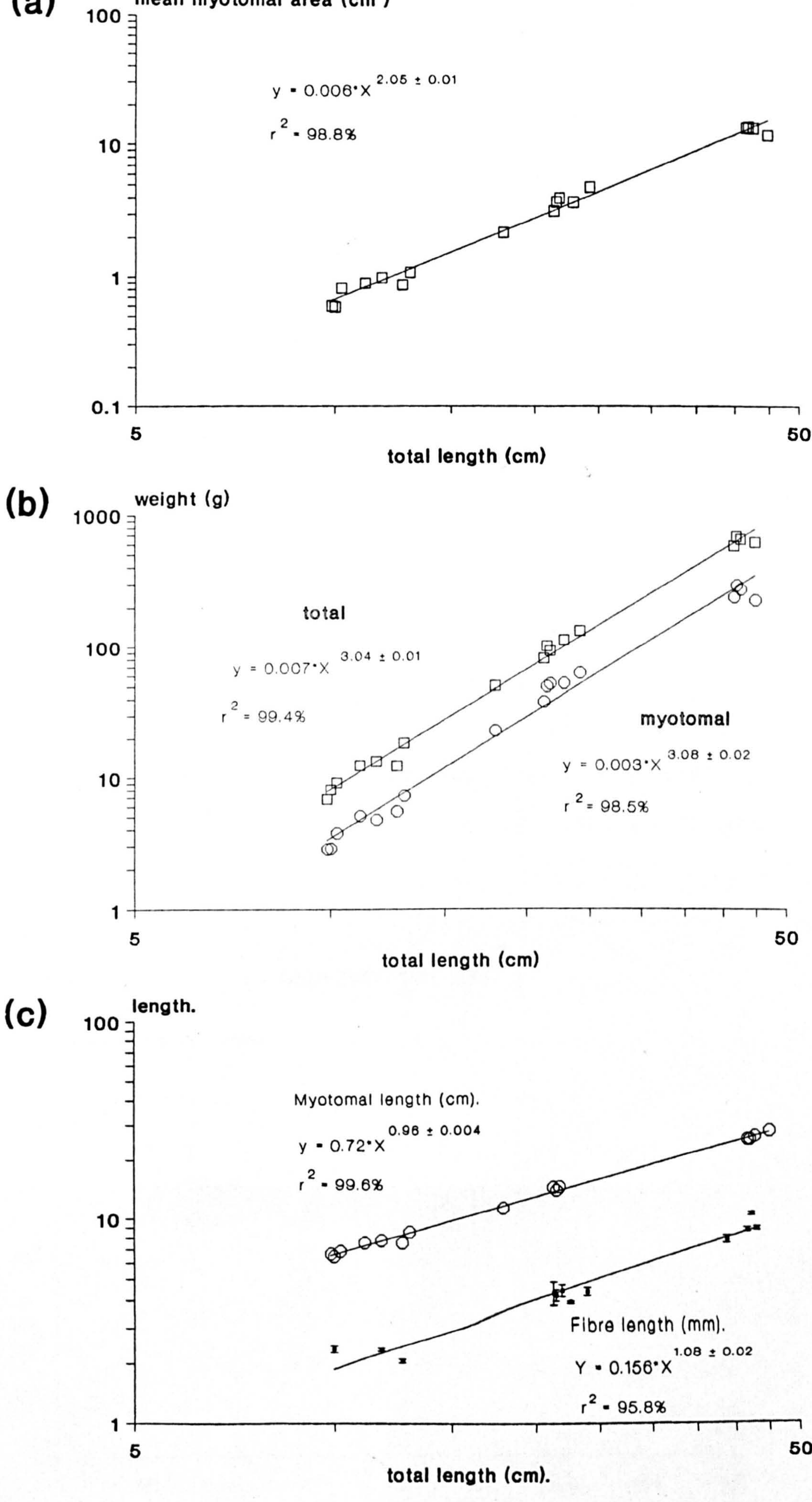

(a)
mean myotomal area (cm)
y = 0.006*X 2.05 ± 0.01
r 2 = 98.8%
100
10
1
0.1
5
50
total length (cm)
(b)
weight (g)
total
y = 0.007*X 3.04 ± 0.01
r 2 = 99.4%
myotomal
y = 0.003*X 3.08 ± 0.02
r 2 = 98.5%
1000
100
10
1
5
50
total length (cm)
(c)
length.
Myotomal length (cm).
y = 0.72*X 0.96 ± 0.004
r 2 = 99.6%
Fibre length (mm).
Y = 0.156*X 1.08 ± 0.02
r 2 = 95.8%
100
10
1
5
50
total length (cm).

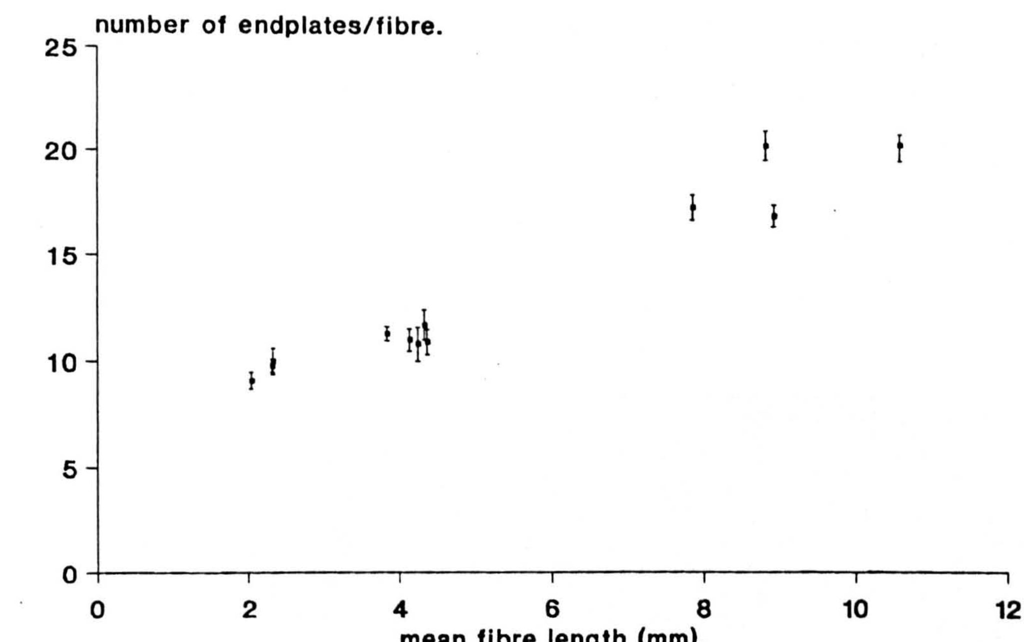
Figure 5.3.

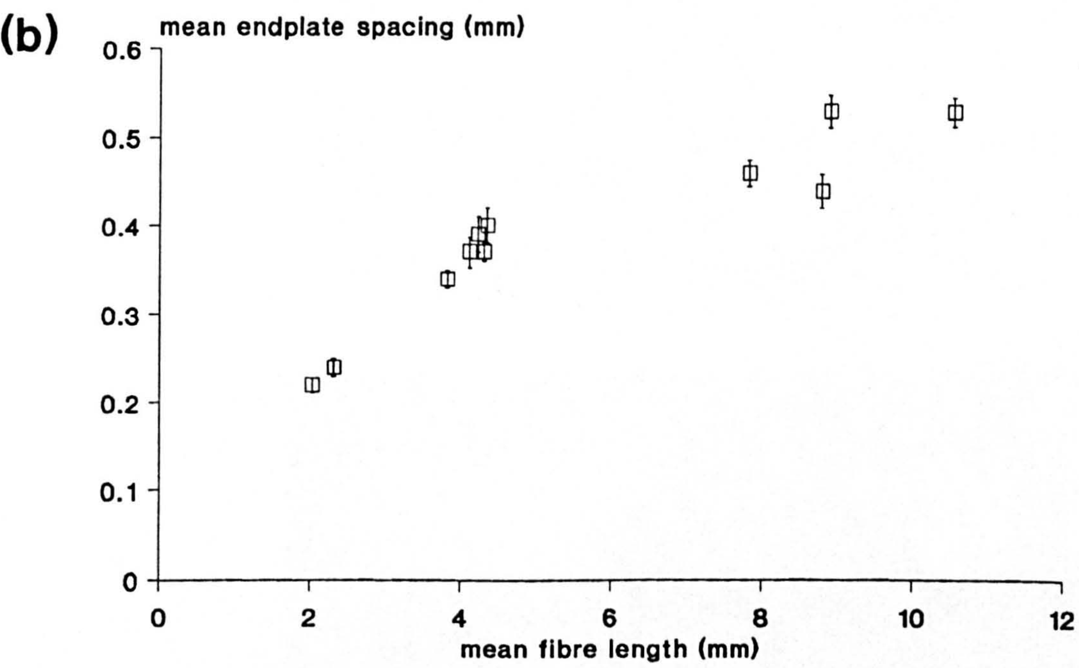
The scaling of the neural innervation of fast muscle fibres from the cod, *Gadus morhua*.

(a). Number of endplates per fibre vs. fibre length. Values are mean ± S.E. of 15–20 fibres from each fish.

(b). Endplate spacing plotted against fibre length. Values are mean ± S.E. from 15–20 fibres per fish.

(c). Plot of the linear relationship between endplate spacing and fibre diameter. Fibre diameters are mean values from 50 fibres per fish. The allometric equation is included in Table 5.1.


(a)
number of endplates/fibre.
25
20
15
10
5
0
0
2
4
6
8
10
12
mean fibre length (mm).


(b)
mean endplate spacing (mm)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
mean fibre length (mm)

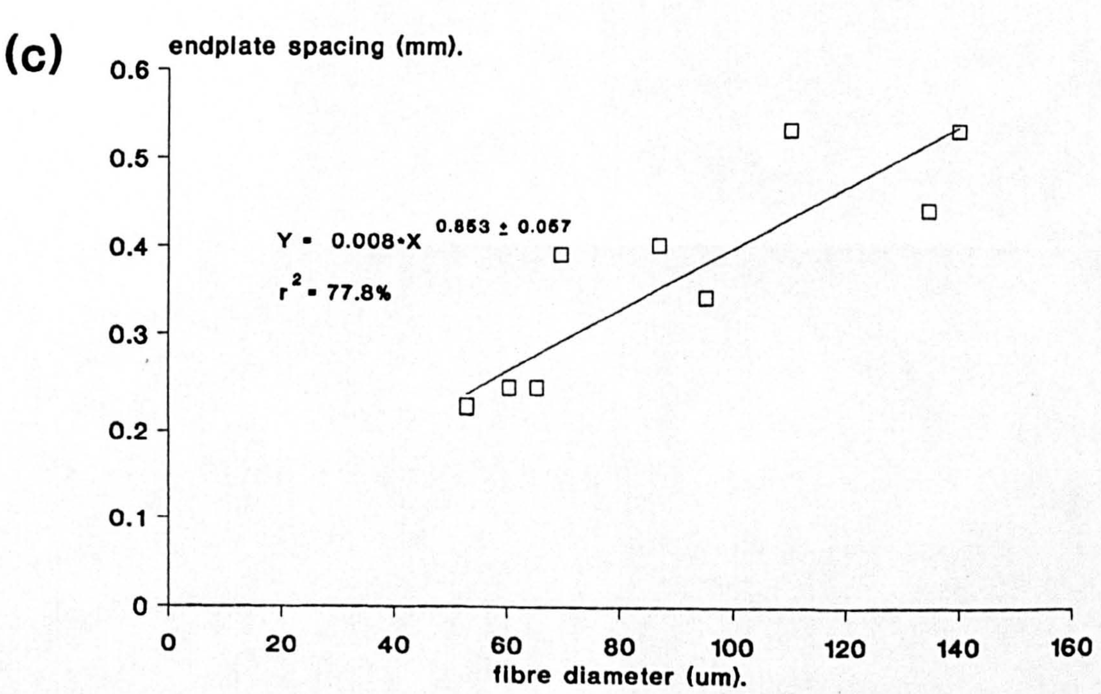

(c)
endplate spacing (mm).
0.6
0.5
0.4
0.3
0.2
0.1
0
Y = 0.008*X 0.853 ± 0.057
r² = 77.8%
0
20
40
60
80
100
120
140
160
fibre diameter (um).

Figure 5.4.

Photomicrographs of teased muscle bundles, illustrating the complexity of the polyneuronal innervation of the fast myotomal fibres of cod, *Gadus morhua* of 91mm total length. (A) scale bar = 0.20mm (B) scale bar = 0.10mm. Preterminal axons and neuromuscular junctions were stained using acetylthiocholine.

(A)

(B)

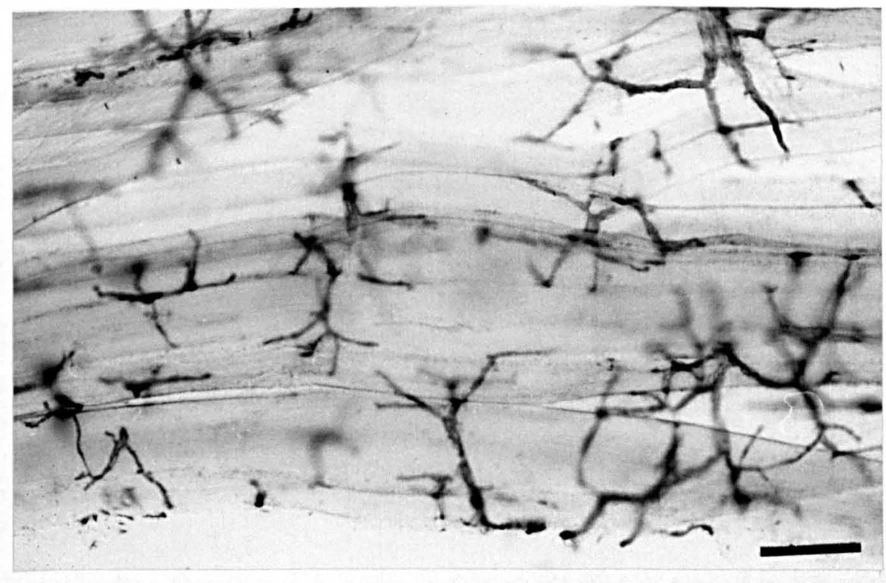


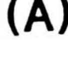
Figure 5.5.

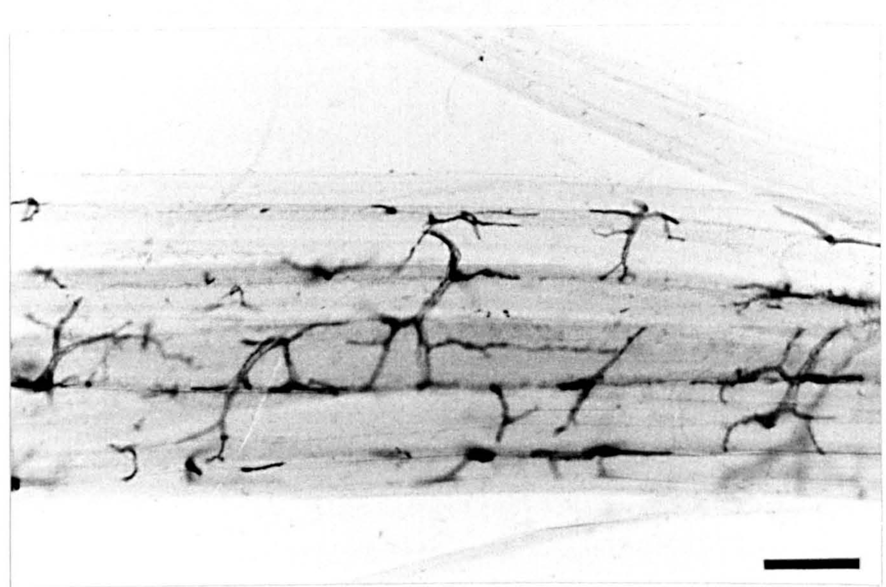
Photomicrographs illustrating the increase in spacing between endplates with growth in cod, *Gadus morhua*:

(A) Juvenile cod, 91mm total length

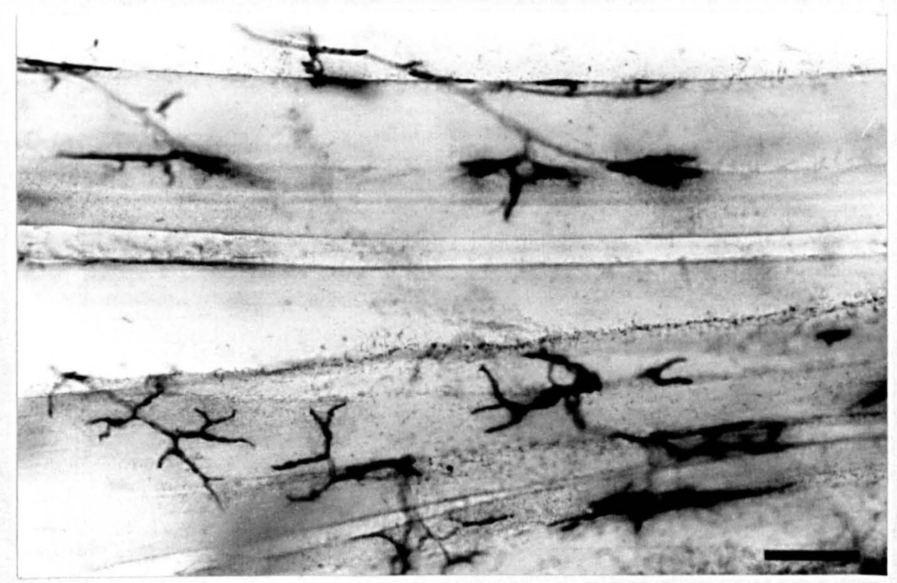
(B) Adult cod, 410mm total length.

Scale bars = 0.10mm.





(B)



CHAPTER 6.

GENERAL DISCUSSION.

Modern biology follows a reductionist trend towards describing organisms in terms of the properties of their component molecules. However, as discussed by Prosser (1986), functions of cells cannot be predicted from the properties of their chemical components and whole organisms are not equal to the sum of the properties of their cells. The more highly integrated a biological system is the more restricted is it's range of tolerance and function.

A decrease in tolerance and range of function, with increased organisation, is illustrated by the thermal tolerance of antarctic fish neural systems. The temperature at which heat blocks propagated action potentials in peripheral nerve is reduced from 40° C in temperate poikilotherms to about 31° C in antarctic fish (Macdonald, 1981). At a higher level of integration, neuromuscular function in *Pagothenia borchgrevinki* is impaired at 12-16° C (Macdonald & Montgomery, 1982). Furthermore, at the whole animal level, notothenioids from McMurdo Sound can only be acclimated for any length of time, to a maximum of 4° C (Somero & DeVries, 1967). A holistic view would consider that determination of the extent of evolutionary adaptation can only really be assessed at the whole animal level. This discussion will focus on the merits and disadvantages realised during this research, of examining adaptation at different levels in the hierarchy of animal organisation.

Muscle contraction and locomotion are of particular theoretical interest in studies of cold adaptation because they involve very high ATP flux. Most tissues exhibit small percentage changes in rates of ATP hydrolysis. In contrast,

during transition from rest to work, the rate of ATP turnover (μmol $g^{-1} min^{-1}$) can increase by orders of magnitude in muscle (Hochachka, 1985). To accommodate such fluxes during locomotion requires an elevation of the rate of respiration. For instance in juvenile salmon, swimming at maximum sustainable speeds involves a 10-12 fold increase in oxygen consumption above resting levels (Brett, 1964).

Muscle metabolism and swimming performance.

At the molecular level, activities of key enzymes involved in aerobic energy supply to the slow muscle of antarctic fish appear to be fully compensated to low temperatures (Walesby & Johnston, 1980; Johnston & Harrison, 1985). In contrast, *N. neglecta* (Dunn & Johnston, 1986), *C. aceratus* (Johnston, 1987) and *P. borchgrevinki* (Davison *et al.* 1988) exhibit an inability to generate energy for propulsion by anaerobic fermentation of glucose. This has been linked to a supposed low level of temperature compensation of high speed swimming performance in antarctic fish (Davison *et al.* 1988). However, examination of muscle metabolism in *N. gibberifrons* has illustrated that not all notothenioids have low activities of glycolytic enzymes (Chapter 2). The low endurance capability at high speeds reported in *P. borchgrevinki*, is therefore not a limitation induced by low temperature. Instead, it results from a reliance on phosphocreatine hydrolysis for anaerobic energy supply. Analysis of enzyme activity of warm-water fish, of similar ecology and swimming behaviour to the notothenioids, is needed to

demonstrate that the energy supply pathway is dictated by swimming behaviour rather than low temperatures.

Muscle structure and swimming performance.

The principle of symmorphosis (Taylor & Weibel, 1981) predicts that the functional and structural design parameters that determine oxygen consumption and flow at each step are just sufficient to provide oxygen flow at the maximal rate. Ultrastructural studies of the slow muscle in antarctic fish demonstrate that the surface density of cristae alters between species (Chapter 4). Therefore, in contrast to the case in mammals, mitochondrial volume density can not be used as an estimate of the concentration of respiratory chain enzymes/aerobic capacity in the muscle cells of antarctic fish. This lack of correlation between mitochondrial volume density and aerobic capacity is further emphasised by studies of the activity of aerobic enzymes in notothenioids. For example, the slow fibres of *C. aceratus* contain higher mitochondrial volume densities than homologous fibres from *N. gibberifrons* (Chapter 4) and yet have similar activities of aerobic enzymes (Johnston, 1987).

Extrapolation of structural parameters to whole animal aerobic swimming performance requires more than estimates of muscle fibre aerobic capacity. Notothenioid slow muscle has relatively high mitochondrial content and enzyme activities that appear to be fully compensated to low temperature (Johnston & Harrison, 1985; Dunn, 1988). However, the myotomes of notothenioids generally contain

relatively low proportions of slow muscle (Johnston *et al.* 1988; Chapter 2). In the majority of cases sustained swimming involves labriform motion, powered by the pectoral fin muscles. Labriform motion is relatively slow (Forster *et al.* 1987; Chapter 3) indicating only a modest capacity for sustained swimming in notothenioids.

<u>Muscle contractile properties and swimming performance.</u>

The difficulties of extrapolating *in vitro* isolated muscle performance to swimming, has been discussed previously (Chapters 3 & 5). The contractile properties of demembranated muscle fibres are obviously relatively unrelated to *in vivo* muscle function. However, such studies have thrown light upon the plasticity of the cross bridge cycle. Possible alterations in the cross bridge cycle may enhance performance at low temperature in antarctic fish muscle (Altringham & Johnston, 1986). Skinned fibre preparations also allow investigation of the energetics of contraction. Fast muscle fibres from notothenioids generate twice as much tension per ATP hydrolysed than homologous fibres from warm-water fish at their preferred body temperature (Altringham & Johnston, 1986; Harrison; 1988). The high economies of contraction for notothenioids may be an example of the energetic advantages of life at low temperature suggested by Clarke (1980).

Live fibre preparations of fish generate almost twice the isometric tensions and have twice the contraction velocities of skinned fibres (Altringham & Johnston, 1988; Curtin & Woledge, 1988). Measurements of power output from these preparations provide a more physiological estimate of

in vivo performance than skinned fibres. As described by Johnston and Altringham (1988), attempts are being made to mimic muscle fibre contraction *in vivo*, using live fibre preparations from fish. Sinusoidal length changes can be imposed on isolated muscle fibres, at frequencies and amplitudes appropriate to locomotion (Josephson, 1985). A large number of variables affect power output in such systems. These include the number of stimuli per cycle, the phase relation of stimuli and length change during a cycle and the size of the length change (Altringham, pers. comm).

The sinusoidal length changes of fish muscle were initially described from detailed kinematic studies of continuous swimming in saithe (Hess & Videler, 1984). Future attempts to model muscle fibre function *in vivo*, in isolated preparations, could possibly involve a combination of kinematic and muscle mechanic techniques. Such techniques could be used to more accurately assess the temperature compensation of muscle performance in antarctic fish.

However, the most conclusive evidence of temperature compensation of locomotory performance involves the study of maximum swimming speeds (Chapter 3). Maximum swimming speeds in juvenile *N. neglecta* appear to be limited, in particular by relatively low tail beat frequencies. This suggests limited compensation in the rates of muscle contraction\relaxation at the higher tail-beat frequencies of smaller antarctic fish. Further studies of maximum swimming speeds in temperate and tropical species are needed for comparison. Techniques described in chapter 3,

demonstrate a conceptual framework by which maximum swimming speeds can be measured.

The importance of scaling in relation to temperature adaptation.

This study has illustrated alterations in muscle composition, contractile properties and swimming performance in fish at different stages of growth (Chapters 2,3 & 6). Any future study of adaptation to temperature should take into account the importance of scaling to biological rate processes. An issue that has surfaced in studies of swimming performance in *N. neglecta*, is the increased constraint that low temperature has on the higher rates of tail-beat frequency in smaller fish (Chapter 3).

Fish larvae are able to swim at very high tail-beat frequencies. For example, larvae of *Engraulis mordax* and *Pleuronectes platessa* exhibit frequencies of over 50Hz (Hunter, 1972) and 30Hz (Batty, 1981), respectively. If the rate processes governing tail-beat frequency are limited by the low temperatures of the antarctic, it will be most obvious in larvae. Future studies of temperature adaptation in muscular systems may benefit from the study of larval stages. Such studies could employ techniques of silhouette photography of swimming performance in fish larval stages (Batty, 1981). The muscle fibre distribution and pelagic lifestyle of notothenioid larvae (Chapter 2) is also common among many temperate water fish (O'Connell, 1981; Batty, 1984; El-Fiky et al. 1987; Blaxter, 1988). This would increase the relevance of comparative studies of swimming performance.

In a similar manner, specific metabolic rates are higher in smaller fish (Brett & Glass, 1973; Weiser, 1985, Weiser & Forstner, 1986). The controversy over the concept of metabolic cold adaptation in fish could possibly be solved by respiratory studies of larval stages. However, there are some problems associated with studies of larval fish. Body shape changes rapidly, external structures including fins are added and modified at different rates and internal organs are appearing or being elaborated at different ages in different species (Blaxter, 1988). Comparative studies between species would have to take such factors into account.

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